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MATHEMATICAL TABLES

A list of the mathematical tables now obtainable from the National Bureau of Standards is printed on page 3 of the Journal cover. New tables will be announced from time to time as they become available.

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RESEARCH PAPER RP1398

Part of Journal of Research of the National Bureau of Standards, Volume 27, July 1941

ENZYMATIC HYDROLYSIS OF DISACCHARIDES AND HALOGENOSALICINS

By William Ward Pigman

ABSTRACT

In agreement with the Weidenhagen theory, the enzymes of almond emulsin hydrolyze all of the disaccharides with β-glucosidic linkages which have thus far been tried. Measurements of rate of hydrolysis are reported for the first time for gentiobiose, 4-glucosidomannose, and lactositol, and comparable data are assembled for other disaccharides. It is shown that very small changes in the configuration or structure of the aglucon sugar have a large influence on the rate of enzymatic hydrolysis. This influence is explained on the basis that two areas of the enzyme are involved in the adsorption of the disaccharides by the enzyme and that the component monosaccharide residues are adsorbed separately on the two areas of the enzyme.

Rates of enzymatic hydrolysis under the standard conditions of Helferich are reported for the *p*-chloro-, bromo-, and iodosalicins. It is shown that the introduction of a halogen in the para position of the salicin aglucon reduces the rate of hydrolysis to less than one-third of the salicin value. While the three halogenosalicins do not differ greatly in their rate of hydrolysis, their relative ease of enzymatic splitting is iodo->bromo->chloro-.

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I. INTRODUCTION

The disaccharides are special types of glycosides for which the aglucon group is a sugar rather than a phenol or alcohol. According to the Weidenhagen theory [1],[1] an enzyme which hydrolyzes a glycoside (e. g., a glucoside) should also hydrolyze disaccharides with the same configuration for the glycosidic linkage (alpha or beta) and with the same sugar (e. g., glucose) for the glycosyl portion of the molecule. In a previous paper [2], the results of a study of the action of almond emulsin on the synthetic disaccharides neolactose (4-β-galactosido-*d*-altrose) and lactulose (4-β-galactosido-*d*-fructose) were reported, and evidence was presented that the same enzyme (β-galactosidase) is responsible for the hydrolysis of these disaccharides

[1] Figures in brackets indicate the literature references at the end of this paper.

and for that of lactose. The earlier work of Helferich, Gootz, and Sparmberg [3] indicated that β-galactosidase hydrolyzes lactose as well as the β-galactosides and that β-glucosidase hydrolyzes cellobiose (4-β-glucosido-*d*-glucose) and the β-glucosides. More recently, Richtmyer and Hudson [4] have measured the rate of hydrolysis of celtrobiose (4-β-glucosido-*d*-altrose), and it would seem probable that the hydrolysis of this disaccharide also is brought about by β-glucosidase.

As it seemed desirable to extend the measurements to other disaccharides with beta linkages, the hydrolysis of gentiobiose (6-β-glucosido-*d*-glucose) and 4-β-glucosido-*d*-mannose was investigated. Although these disaccharides are known to be hydrolyzable by the enzymes of almond emulsin, the earlier measurements were not carried out under conditions comparable to those used for the previously mentioned disaccharides. Through the courtesy of M. L. Wolfrom, who provided the material for the investigation, it has been possible to investigate the enzymatic hydrolysis of a derivative of lactose, lactositol (4-β-glucosido-*d*-sorbitol). This compound is similar in structure to lactobionic acid, the enzymatic splitting of which has been reported by Helferich, Pigman, and Isbell [5], and differs from the lactobionic acid only in having a primary alcoholic group instead of a carboxyl group. The present investigation also includes the results of a study of the rate of enzymatic hydrolysis of the *p*-iodo-, bromo- and chlorosalicins. The preparation and optical rotation of these substances were previously reported by D. H. Brauns [6], who very generously made these substituted salicins, as well as several disaccharides, available for this investigation. These halogenosalicins are apparently the first aromatic glycosides to be investigated which contain halogen atoms in the aglucon. The enzymatic studies of the substances included in this investigation, in conjunction with the earlier obtained results, make it possible to investigate the effect of changes in the configuration and structure of the aglucon on the enzymatic action and to test further the validity of the Weidenhagen concept.

II. DISCUSSION OF RESULTS

In table 1 are summarized the results obtained for the disaccharides and derivatives. The first group of disaccharides, as previously mentioned, may be considered as β-glucosides in which the aglucon is a second hexose molecule. The second group of compounds are, similarly, β-galactosides in which the aglucon is a sugar or sugar derivative.

The relative ease of hydrolysis of the various substances is indicated by a quantity which has been called the "enzyme efficiency" (*EE*). This quantity is a function of the velocity constant for fixed conditions of substrate concentrations (0.052 *M*), pH (5.0), temperature (30° C), and enzyme concentration. Helferich, who originated this method of expression of ease of hydrolyzability, calls this quantity the "Wertigkeit."

All of the compounds listed in table 1 are split by almond emulsin, in agreement with the Weidenhagen theory. All members of the first group of compounds probably are hydrolyzed by the same β-glucosidase and the members of the second group by the same β-galactosidase.

TABLE 1.—*Hydrolysis of disaccharides by enzymes of almond emulsin (Rohferment)*

Disaccharide or derivative	Aglucon	Point of attachment in aglucon	Enzyme efficiency (EE) $\times 10^3$	Reference
β-GLUCOSIDES				
Cellobiose	*d*-Glucose	4	[a] 159, 180	[8]
Gentiobiose	do	6	[c] 75	
4-β-Glucosidomannose	*d*-Mannose	4	[c] 2.3	
Celtrobiose	*d*-Altrose	4	[c] (23)	[4]
β-GALACTOSIDES				
Lactose	*d*-Glucose	4	[a] 11.2, 11.4	[5]
Lactulose	*d*-Fructose	4	[b] (14)	[2]
Neolactose	*d*-Altrose	4	[b] (2.8)	[2]
Lactobionic acid	*d*-Gluconic acid	4	0.41	[5]
Lactositol	*d*-Sorbitol	4	[c] .84	

[a] The value was calculated from that originally given for a slightly different concentration on the assumption that the ratio of the EE for cellobiose to that for celtrobiose is the same at the 2 concentrations.
[b] Calculated according to the same method as for celtrobiose, using lactose as standard.
[c] These values have been determined in the present investigation.

The pronounced effects of even slight changes in the aglucon are very evident. Thus, cellobiose and 4-β-glucosidomannose differ only in the configuration of one carbon atom, and yet this change from the glucose to the mannose configuration produces a very marked decrease in the rate of the enzymatic cleavage. Similarly, 4-β-glucosido-*d*-mannose and celtrobiose differ only in the configuration of the third carbon of the aglucon sugar, and yet the EE values of the sugars are in the ratio 1:10. A change in the position of the glucosidic bridge from the fourth carbon of the aglucon sugar (cellobiose) to the sixth carbon atom (gentiobiose) with no other changes in configuration or structure reduces the rate of hydrolysis about one-half. For the β-galactosides, corresponding differences may be observed. It is of interest to note for these compounds the effect of breaking the pyranose ring of the aglucon by reduction of the glucose group to the sorbitol group or gluconic acid group. The EE values for lactobionic acid and lactositol are approximately one-tenth to one-twentieth of the values for lactose. As was previously shown, lactulose which probably has fructofuranose [7] as the aglucon sugar is slightly more rapidly hydrolyzed than lactose which has glucose as the aglucon sugar [2].

The magnitude of the effect of small changes in the configuration or structure on the rate of enzymatic hydrolysis may appear to be difficult to explain. However, if we assume,[2] as suggested by Euler [9], that the enzyme adsorbs the substrate material at two positions and not merely at one, as assumed by Weidenhagen and other workers [1], the results are explainable. If each of the hydroxyl groups of the aglucon potentially is adsorbable by the enzyme when steric conditions are favorable, then the change of configuration of one carbon atom of the aglucon sugar would undoubtedly influence the number of adsorption

[2] The suggestion of Euler [9] that the action of enzymes involves an intermediate adsorption of substrate and enzyme *at two positions* has been criticized by Weidenhagen [1] but otherwise has not received much attention. It seems to the writer, however, that such a mechanism is not in contradiction to the Weidenhagen theory [1] or to the modified form previously suggested [10], but instead, the "two affinity theory" is capable of explaining many of the difficulties of the original Weidenhagen theory. At the present time it seems that Euler's concept, when extended and made more specific, provides a logical explanation for the known reactions and properties of the glycosidases and possibly of other enzymes. Additional experimental work is being carried out in order to test the validity of this mechanism.

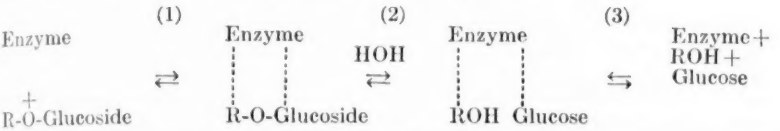
bonds which the aglucon can form with the enzyme. This effect would, however, be much greater than the loss or gain of one linkage, since the change of configuration of one carbon simultaneously would affect the conformation of the entire aglucon group. The various hexose sugars differ in that the different configurations of the carbons of the pyranose ring produce different conformations of the ring as a whole, depending upon the spatial distribution and proximity of the hydroxyl groups [11]. Because of this effect on the conformation of the pyranose ring produced by the change in configuration of even a single carbon atom or by a change in the position or nature of substituent groups, changes in the configuration or structure of a disaccharide aglucon group would undoubtedly produce an effect on the aglucon-enzyme complex much greater than that produced by the loss of a single adsorption bond.

The slow rate of hydrolysis of lactositol and of lactobionic acid is more difficult to understand, since the breaking of the pyranose ring produced by reduction changes the aglucon from a cyclic structure to a branched-chain methylpentanol derivative. The branched chain should be more easily accommodated to the adsorbing atoms of the active centers of the enzyme than a similar cyclic structure. For this reason, it would seem that the lactositol should be more easily hydrolyzed than either lactose or phenyl β-galactoside, but actually the lactose is cleaved about 13 times and the phenyl β-galactoside about 60 times as rapidly as the lactositol under the conditions employed. Although it would be supposed that lactositol is adsorbed well, the rate of hydrolysis is slow. The following considerations indicate, however, that this apparent anomaly is not necessarily in contradiction with the above explanations, which are based on the postulate that ordinarily high adsorption results in rapid enzymatic hydrolysis. For a series of glucosides or galactosides of similar type, the adsorption of the aglucon increases (1) as the number of atoms capable of adsorption is made greater or (2) as the new groups have greater adsorptive power than those replaced. The results obtained with the lactositol would, however, indicate that for such a series having aglucon groups of increasing adsorptive power, the rate of hydrolysis increases at first, reaches a maximum, and then decreases. For the series CH_2OH-$(CHOH)_n$-CH_2-O-glucoside (where n increases from zero to an indefinitely large number) it would seem that the rate of hydrolysis by the enzyme should reach a maximum at some intermediate value of n and then decrease as the chain is lengthened.

The substituted-phenyl β-glucosides or galactosides probably constitute a series for which the increase of adsorption is dependent on the adsorptive powers of the substituent groups, although the electronic effects of these groups probably cannot be completely disregarded. The enzymatic hydrolysis of the substituted-phenyl β-glucosides has been studied for a large number of compounds by Helferich and coworkers [12, 13, 14]. Of the compounds studied, Helferich reports that all having groups in the ortho position are more easily hydrolyzed than the unsubstituted phenyl β-glucoside except the o-NH_2-CH_2-phenyl β-glucoside. The galactosides, although not so well studied, behave similarly. But from the results of Myrbäch's work [15] on the action of amines on invertase, it would be expected that the amino group would be readily adsorbed on the enzyme surface and even possibly form compounds of the Schiff

base type with carbonyl groups in the active adsorbing area. The results reported for the aminophenyl glycosides are then in agreement with those reported for lactositol in that in both cases while the aglucon is probably highly adsorbed the rate of hydrolysis is slow.

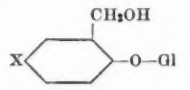
The following mechanism for the enzymatic hydrolysis of a glycoside is advanced to explain this apparently unusual combination of high adsorption of the glucoside and slow rate of hydrolysis. At the same time it appears to be in qualitative agreement with most of the data obtained by other investigators in this field.



In the above equations, the dotted lines represent adsorption forces which may be hydrogen bonds or Van der Waals forces. There may be one or more for the aglucon group and for the glucoside group. The number of bonds depends on the number of atoms in the glucoside (including the aglucon) capable of forming such bonds and also on the arrangement of the bond-forming atoms of the active areas of the enzyme. Ordinarily reaction (1) or (2) is the rate-determining reaction, but when the aglucon is highly adsorbed, the ROH-enzyme-glucose compound dissociates slowly and the rate-determining reaction seems to be reaction (3). This mechanism will be developed further in later publications.

In table 2 are listed the *EE* values (enzyme efficiency) for the *p*-chloro-, *p*-bromo- and *p*-iodosalicins. The three substances are hydrolyzed at approximately the same rates, but the *EE* values are in the order: iodosalicin>bromosalicin>chlorosalicin. All three substances are more slowly hydrolyzed than salicin. The substitution of a halogen atom into the para position of salicin lowers the rate of hydrolysis.

TABLE 2.—*Summary of action of almond emulsin on halogenosalicins*



Substrate	Structure of glucoside	Enzyme efficiency (*EE*)	*EE* (dilute concentration)
Salicin	*X*=H	2.0	2.9
p-Chlorosalicin	*X*=Cl	0.48	0.99
p-Bromosalicin	*X*=Br	.60	
p-Iodosalicin	*X*=I	(.62)	1.24

III. EXPERIMENTAL PROCEDURE

1. SUBSTRATES AND SUBSTRATE SOLUTIONS

In general, the substrate concentrations in the reaction mixture were the same as those ordinarily used by Helferich (0.052 *M*). Since the *p*-iodosalicin is too insoluble for the customary concentration to be used, the measurements were made on solutions of half the normal concentration. The rotations and concentrations of the disaccharides used are given in table 3. The salicin derivatives had the rotations previously reported by D. H. Brauns [6].

TABLE 3.—*Optical rotation and concentration of disaccharides used in the investigation*

Compound	$[\alpha]_D^{20}$	Grams of substrate in 25 ml buffer solution
Lactose . H_2O	52.6	0.703
4-β-glucosido-*d*-mannose . H_2O	5.2	.704
Cellobiose . H_2O	34.8	.703
Gentiobiose	9.7	.668
Lactositol . $2H_2O$	13.3	.743

The concentrations given in table 3 are not the concentration in the reaction mixture but of a stronger solution which was later diluted with enzyme solution in the ratio of 2 volumes of substrate solution plus 1 volume of enzyme solution. The quantities indicated in the table 3 were made up to a volume of 25 ml with Walpole 0.2-*M* acetate buffer (pH 5.0 at 18° C.).

2. ENZYME SOLUTIONS

The enzyme solutions were made up as previously described [16] from a sample of almond emulsin (Rohferment) furnished by the courtesy of B. Helferich. The material had a β-glucosidase value [17] of 1.0. For the enzyme concentrations, see tables 4 and 5.

3. RATE OF HYDROLYSIS MEASUREMENTS

The details of the method used have been previously described [16]. For salicin and the halogenosalicins, the rate of hydrolysis was determined polarimetrically. To 2 ml of the buffered substrate solution there was added 1 ml of the enzyme solution. After t minutes at 30° C, the reaction was stopped by the addition of 0.2 g of potassium carbonate, and after about 1 hour the solution was read in a 2-dm tube at 20° C. For the experimental results, see table 4.

TABLE 4.—*Enzymatic hydrolysis of halogenated salicin*

[T=30° C; pH=5.0; g=0.0240 g of enzyme in 50 ml of reaction mixture]

Time, t (min)	°S[a] after t min	Hydrolysis (percent)	[b]$k\times10^4$	Substrate concentration (M)	EE
SALICIN					
0	−5.76				
10	−3.42	27.2	138	0.052	1.91
12	−3.09	31.0	135	.052	1.87
15	−2.08	42.7	161	.052	2.23
∞	+2.83			Avg...	2.0
0	−2.87				
9.0	−1.38	34.7	206	.026	2.85
10.0	−1.21	38.7	213	.026	2.94
∞	+1.42			Avg...	2.9
BROMOSALICIN					
0	−6.90				
30.1	−4.15	28.3	47.9	0.052	0.66
41.4	−3.86	31.2	39.3	.052	.54
∞	+2.83			Avg...	0.60
CHLOROSALICIN					
0	−6.48				
30.0	−4.36	22.8	34.1	0.052	0.48
40.0	−3.95	27.2	34.4	.052	.47
∞	+2.83			Avg...	0.48
0	−3.29				
20.0	−1.81	31.4	81.9	.026	1.13
40.3	−1.25	43.3	61.2	.026	0.85
∞	+1.42			Avg...	0.99
IODOSALICIN					
0	−3.39				
20.4	−1.55	38.3	103	0.026	1.42
41.0	−0.92	51.4	76	.026	1.06
∞	+1.42			Avg...	1.24

[a] Measured in 2-dm tube with a Bates saccharimeter. A small correction for the rotation of enzyme (−0.17) has been made.

[b] Common logarithms.

TABLE 5.—*Enzymatic hydrolysis of disaccharides*

[T=30.0° C; pH=5.0]

Time (min)	0.1 N [a] I_2 used (ml)	I_2 used, corrected [b] (ml)	Hydrolysis (percent)	[c] $k\times10^5$	[d] g	[e] $EE\times10^3$
LACTOSE						
0	10.59	0	-------	-------	-------	-------
104.5	16.06	2.26	21.3	100	0.3053	10.9
157.2	17.08	3.28	31.0	103	.3053	11.2
236	18.42	4.62	43.6	105	.3053	11.4
∞	---------	(10.59)	(100)	--------	Avg----	11.2
4-β-GLUCOSIDO-*d*-MANNOSE						
0	9.61	0	-------	-------	-------	-------
1,596	17.47	4.97	51.7	19.8	0.3053	2.15
1,685	18.18	5.68	59.1	23.0	.3053	2.50
∞	---------	(9.61)	(100)	--------	Avg----	2.33
GENTIOBIOSE [f]						
0	10.00	0	0	-------	-------	-------
75.0	15.08	3.80	38.0	277	0.1204	76.4
125.3	16.66	5.38	53.8	268	.1204	74.0
∞	---------	(10.00)	(100)	--------	Avg----	75.2
CELLOBIOSE						
0	9.67	0	-------	-------	-------	-------
20.0	13.42	2.26	23.4	579	0.1198	161
33.0	14.36	3.20	33.1	529	.1198	147
60.3	16.68	5.52	57.1	610	.1198	169
∞	---------	(9.67)	(100)	--------	Avg----	159
LACTOSITOL						
0	0.59	0	-------	-------	-------	-------
1,580	6.58	2.78	26.7	8.5	0.3053	0.92
3,010	7.93	4.13	39.7	7.3	.3053	.79
4,540	9.39	5.59	53.7	7.4	.3053	.81
∞	---------	(10.41)	(100)	--------	Avg----	0.84

[a] The volume of the reaction solution was 10 ml for the lactose, gentiobiose, and lactositol experiments and 9 ml for the others.

[b] This column gives the value after correction for the amount of iodine absorption by the amount of sugar initially present (the value at zero time) and for the amount of iodine absorbed by the enzyme under similar conditions. For the lactose and lactositol, 3.33 ml of enzyme solution required 3.21 ml of 0.1 N iodine; for the 4-β- glucosido-*d*-mannose experiments, 3 ml of enzyme required 2.89 ml; for the cellobiose experiments, 3 ml required 1.49 ml; and for the gentiobiose experiments, 3.33 ml of enzyme solution required 1.28 ml of 0.1 N iodine.

[c] Calculated, using common logarithms.

[d] Grams of enzyme in 50 ml of reaction mixture.

[e] Enzyme efficiency, or Wertigkeit. $EE=k/(g\times\log 2)$.

[f] For gentiobiose, the slightly low initial value indicates that the material was not entirely free from methyl alcohol. Gentiobiose crystallizes from methyl alcohol with 2 moles of methyl alcohol. This alcohol of crystallization is lost very easily by keeping the compound in a desiccator over calcium chloride.

The hydrolysis of the disaccharides was measured by determining the aldose sugar present at any time by Helferich's modification of Cajori's iodometric method [16]. Since the reaction takes place stoichiometrically, the amount of iodine required for 100-percent hydrolysis would be twice that for the original disaccharide. The experimental results are given in table 5.

The methods used for making the calculations are given in another place [16]. Toluene was added to all experiments to prevent the growth of microorganisms.

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WASHINGTON, May 2, 1941.

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OPTICAL ROTATORY RELATIONSHIPS EXHIBITED BY AROMATIC AND ALIPHATIC GLYCOSIDES

By William Ward Pigman and Horace S. Isbell

ABSTRACT

A comparison of the rotations of a large number of glycosides reveals that aromatic groups in general (phenyl and substituted phenyls) produce rotational effects quite different from those produced by ordinary aliphatic radicals. These influences are manifested in several ways: (1) When an aromatic nucleus is attached to an asymmetric carbon through an oxygen linkage, the rotatory contributions of *other* asymmetric carbons attached to this carbon are greater by a fairly constant amount than they are when the attached group is an aliphatic radical. Using Hudson's concept, this may be expressed by the statement that the $2B$ values for aromatic glycosides are usually 9,000 to 13,000 greater than the $2B$ values for the aliphatic glucosides. (2) Aromatic β-glucosides (substituted-phenyl β-glucosides) usually are considerably more levorotatory than the aliphatic β-glucosides. Phenyl β-glucosides when substituted with "*ortho-para* directing groups" in any position or ordinarily with "*meta* directing groups" in the *ortho* position have molecular rotations in the region of $-17{,}000$ to $-20{,}000$, whereas the molecular rotations of the aliphatic β-glucosides, with the exception of the glucosides of the tertiary alcohols, all fall between $-6{,}500$ and $-9{,}500$. However, "*meta* directing groups" in the *meta* and *para* positions increase the molecular rotations to values greater than $-20{,}500$, and for *p*-nitrophenyl β-glucoside the molecular rotation is $-31{,}130$. An interesting "*diortho* effect", which consists in a marked decrease in the optical rotation caused by the substitution of *two* groups in the *ortho* positions of phenyl β-glucoside, is pointed out. It is shown that in a series of related glucosides, aliphatic or aromatic, the molecular rotations of the β-glucosides are directly proportional to the rotatory contributions of the glucosidic carbons.

A correlation is demonstrated between the molecular rotations of certain substituted-phenyl β-glucosides and the pK values of the corresponding phenols. This relationship is similar to that previously found by Betti for a different series of active compounds. The parallelism between the dissociation constants of the phenols and the optical properties of the corresponding glucosides supports the hypothesis that the optical rotation is conditioned by the same intramolecular electronic forces as those which control the dissociation of the phenolic hydrogen. The effect is presumably exerted through the electrons forming the bond between the phenolic oxygen and the glucosyl group.

Experimental details for the preparation of the phenyl α- and β-*d*-α-glucoheptosides (and pentaacetates), the phenyl α- and β-*d*-α-mannoheptosides, and phenyl α-*d*-taloside (and tetraacetate) are described. The optical rotations and melting points of these new substances are given.

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I. INTRODUCTION

The numerous data available for the optical rotations of the alpha and beta modifications of the phenyl and substituted-phenyl glycosides make possible many interesting correlations between the optical rotations and other properties of the aromatic glycosides. For correlating optical rotation and structure Hudson [1, 2] [1] has expressed several rules. The so-called first rule of isorotation relates to the optical rotation of the glycosidic carbon. If the formulas for α- and β-*d*-glucose are written as ring structures differing solely in the configuration of carbon 1, and if the rotation which is due to carbon 1 is called A, and the rotation which is due to the rest of the molecule is called B, the molecular rotation of one isomer can be represented as $+A+B$ and the rotation of the other isomer as $-A+B$. The sum of the rotations is then $+2B$ and their difference $+2A$. When the molecular rotations of the alpha and beta modifications of substances having like glycosidic groups, like ring structures, and like configurations on the adjacent carbon atoms are compared, it has been observed that the differences ($2A$ values) are nearly constant. This approximate equality is the basis of the first rule of isorotation, which states [2, p. 61] "*the rotation of the carbon 1 in the case of many substances of the sugar group is affected in only a minor degree by changes in the structure of the remainder of the molecule.*" The sum of the molecular rotations of the alpha and beta sugars, $+2B$, varies from sugar to sugar; but if the sums of the molecular rotations of the sugars are compared with the sums of the molecular rotations of the methyl glycosides, it will be observed that the values of $2B$ obtained for the sugars are close to the values of $2B'$ obtained for the corresponding methyl glycosides. This is the basis for Hudson's second rule of isorotation which states [2, p. 63] "*changes in the structure of carbon 1 in the case of many substances of the sugar group affect in only a minor degree the rotation of the remainder of the molecule.*"

Hudson has investigated the effect of variations in the glycosidic aglucon group on the values of B and has shown that changes in the structure of the aglucon group produce only small effects on the rotatory contributions of the remaining carbon atoms ($2B$). But as pointed out by several authors [3, 4], the value of $2B$ obtained for the phenyl α- and β-α-glucosides differs considerably from the value obtained for the methyl α- and β-α-glucosides and related substances. According to one of the current theories for the origin of optical rotation [5], the introduction of a chromophoric group at one point in an optically active substance induces a change in the optical rotation of

[1] Figures in brackets indicate the literature references at the end of this paper.

the rest of the molecule. The chromophoric group is particularly influential when adjacent to an asymmetric center, as for example, in the benzyl glucosides.

The preparation of numerous substituted-phenyl α- and β-glycosides, particularly by Helferich and coworkers [6] as well as others, makes possible the comparison of the optical rotations of a wide variety of aromatic glycosides. Such comparisons furnish information concerning the effect of substitution in the benzene ring on the optical rotation of the corresponding glycosides, and perhaps on the origin of optical rotation.

II. COMPARISON OF OPTICAL ROTATIONS OF GLYCOSIDES

1. 2*B* VALUES FOR AROMATIC AND ALIPHATIC GLYCOSIDES

In table 1 are recorded the values for 2*A* and 2*B* for the aromatic and aliphatic glucosides, galactosides, mannosides, arabinosides, and rhamnosides and their acetylated derivatives for which the optical rotations of both the alpha and beta isomers have been found in the literature. The phenyl *d*-α-glucoheptosides and *d*-α-mannoheptosides are new compounds reported in this paper.

The 2*B* values for the aliphatic and aromatic glycosides of the same sugar differ by significant amounts. The alkyl glucosides have an average value for 2*B* of 23,200, while the phenyl, nitrophenyl, and *p*-hydroxyphenyl glucosides average 32,160, or almost 40 percent more. Inspection of table 1 reveals that the 2*B* values for the aromatic and aliphatic glycosides of arabinose, galactose, *d*-α-glucoheptose, *d*-α-mannoheptose, *d*-mannose, and *l*-rhamnose also differ by substantial amounts. It is interesting to note that the benzyl glucosides have a value for 2*B* slightly lower than the normal value for the aliphatic series. The introduction of a methylene (CH_2) group between the aromatic nucleus and the asymmetric carbon reduces the effect of the phenyl group, so that the benzyl glucosides have a value near that for the aliphatic series.

It may also be seen from table 1 that the same qualitative differences are observable for the acetylated glycosides. The most striking effect is shown by the acetylated *d*-α-glucoheptosides, for which the difference between the 2*B* values for the phenyl and methyl glycosides is about 47,000, or more than 100 percent greater than the 2*B* value for the methyl *d*-α-glucoheptosides.

TABLE 1.—*Sums and differences of molecular rotations of alpha and beta pairs of sugars, glycosides, and acetylated derivatives* [a]

Substances	Not acetylated		Acetylated	
	Rotations measured in H_2O for *D* line		Rotations measured in $CHCl_3$ for *D* line	
	Difference, [b] $2A$	Sum, [c] $2B$	Difference, [b] $2A$	Sum, [c] $2B$
l-Arabinose	17,050	40,170	33,300	60,300
Methyl *l*-arabinosides	37,460	43,140		
Ethyl *l*-arabinosides	39,040	44,180		
Phenyl *l*-arabinosides	53,610	56,330		
d-Galactoses	17,640	36,660	32,790	50,750
Methyl *d*-galactosides	38,220	37,940	53,270	43,130
Phenyl *d*-galactosides	65,810	45,410	74,780	74,180
o-Tolyl *d*-galactosides	62,510	39,100	77,590	74,090
d-α-Glucoheptoses			38,010	42,450
Methyl *d*-α-glucoheptosides	41,790	8,210	53,600	39,700
Phenyl *d*-α-glucoheptosides	72,340	20,980	78,930	86,870
d-Glucoses	16,840	23,580	38,220	41,180
Methyl *d*-glucosides	37,500	24,220	54,870	40,690
Ethyl *d*-glucosides	39,290	24,010	58,250	41,170
Propyl *d*-glucosides	39,890	22,690		
n-Hexyl *d*-glucosides			59,030	41,810
Ethylene glycol *d*-glucosides	37,240	23,520		
Allyl *d*-glucosides	38,290	19,710		
1,3-Propane glycol *d*-glucosides	41,380	24,140		
Cyclohexyl *d*-glucosides	45,800	24,080	62,620	42,140
Benzyl *d*-glucosides	49,870	20,950	85,750	39,890
Phenyl *d*-glucosides	67,780	31,400	80,640	61,960
p-Nitrophenyl *d*-glucosides	95,800	33,740		
p-Aminophenyl *d*-glucosides	[d] 70,280	[d] 35,020		
p-Hydroxyphenyl *d*-glucosides	65,910	31,330		
p-Benzoylphenyl *d*-glucosides			82,860	67,080
d-α-Mannoheptoses	17,730	37,030		
Phenyl *d*-α-mannoheptosides	70,650	47,870		
d-Mannoses	8,340	2,220	31,310	11,630
Methyl *d*-mannosides	28,930	1,830	36,050	−470
Phenyl *d*-mannosides	47,430	10,730	57,970	4,750
l-Rhamnoses	7,870	4,730		
Methyl *l*-rhamnosides	28,140	5,860	[e] 30,250	[e] −2,430
Phenyl *l*-rhamnosides	46,490	−4,450	48,540	−10,080

[a] Compiled from the data listed in table 5, p. 20.
[b] The differences were obtained by subtracting the molecular rotation ([α]×mol. wt.) of the isomer having the smallest dextrorotation from the isomer having the largest dextrorotation. In a number of cases the temperature at which the rotations were measured was slightly greater or less than 20° C.
[c] The algebraic sum of the molecular rotations of the alpha and beta isomers of the substances.
[d] One rotation was measured in methyl alcohol.
[e] Rotations measured in acetylene tetrachloride solution.

2. RELATION BETWEEN THE VALUES OF 2*A* OF GLUCOSIDE PAIRS AND THE MOLECULAR ROTATIONS OF THE CORRESPONDING β-*d*-GLUCOSIDES

In accordance with the "isorotation concept" the molecular rotation of a β-*d*-glucoside may be represented by the equation $M=-A+B$. Hence, if the molecular rotations of a series of glucosides are plotted against the corresponding values for A, the points should lie on a straight line having a slope of -1 and with the intercept on the M axis having the value represented by B. If $2A$ is plotted against M, the slope will be 1/2 and the intercept again will be B. In the preceding section it was shown that the value of B for the aliphatic glucosides is less than that of B for the aromatic glucosides, and since the slopes

are the same but the intercepts different, the two series should be represented by two parallel lines.

In figure 1, the two lines were constructed from the values of B for the aromatic and aliphatic series. These values are represented by the squares at the left of the figure. From these squares, straight lines were constructed with a slope of $-1/2$. The values of $2A$ for all of the glucoside pairs which could be found in the literature were then plotted against the molecular rotations of the corresponding β-*d*-glucosides.

All of the points except that representing the benzyl glucosides, which is represented by the triangle, fall close to the two lines which were drawn in conformity with Hudson's isorotation equation, modi-

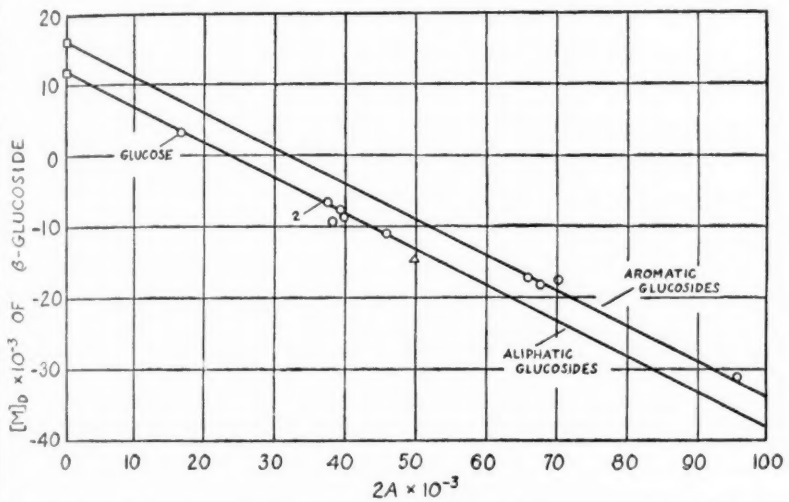


FIGURE 1.—*Molecular rotations of β-d-glucosides and the corresponding rotatory contributions (2A) of the glucosidic carbon.*

fied so that B has a different value for aromatic glucosides from that for the aliphatic glucosides. Since the benzyl glucosides are of mixed aromatic and aliphatic character, a deviation is not surprising, yet the direction of the deviation is different from that which might have been expected from the mere proximity of the phenyl group.

On the basis of the regularity in the optical rotations, illustrated in figure 1, it may be assumed that in a series of closely related α- or β-*d*-glucosides the changes in optical rotation produced by a change in the aglucon group arise in large part from a change in the rotatory contribution of the first carbon of the glucoside. This generalization will be used in the next section of this paper for the purpose of explaining the effects of changes in the aglucon group on the optical rotations of the β-*d*-glucosides. Although in the preceding discussion a sharp difference has been drawn between aromatic and aliphatic glucosides, it seems likely that when the aglucon group of aliphatic glucosides contains carbonyl groups, ethylenic linkages, conjugated systems, etc., deviations might be anticipated. Thus the allocation of the glucosides into classes having closely related aglucon groups is some-

what arbitrary, but it justified because it permits a correlation of the optical rotation of glucosides with the electronic structure of the aglucon.

III. RELATIONS BETWEEN THE STRUCTURE OF THE AGLUCON GROUP AND THE OPTICAL ROTATION

In table 2 are listed the molecular rotations of the aliphatic β-*d*-glucosides. Except for several of the lower homologues, the normal aliphatic series of glucosides appear to have about the same molecular rotations from the propyl to the *n*-hexadecyl (cetyl) glucosides (3 to 16 carbons in the aglucon). It should be noted that from the hexyl to the dodecyl glucosides the rotations are reported for methyl alcoholic solutions. The glucosides of tertiary alcohols have appreciably lower rotations than the normal and iso aliphatieg lucosides. On the other hand the introduction of hydroxy and methoxy groups into the aglucon results in only small changes in the molecular rotations. Double bonds in the β-γ position and carbonyl groups in the beta position of the aglucon produce only slight increases in the rotations as is evidenced by the rotations of the allyl and glycollic acid β-*d*-glucosides. A glucoside with an α-β double bond would have certain similarities to an aromatic glucoside, but apparently such glucosides have not been made.

The molecular rotations of cyclohexyl β-*d*-glucoside and benzyl β-*d*-glucoside are higher than those of the aliphatic series but are lower than the molecular rotation of phenyl β-*d*-glucoside. When the phenyl group is situated at a greater distance from the first asymmetric carbon atom, as in the case of the phenylethyl and phenylpropyl glucosides, it loses its marked influence and the rotations of these glucosides are in the range of the rotations of the aliphatic glucosides.

As may be seen from a comparison of the values given in tables 2 and 3, the optical rotations of aromatic β-*d*-glucosides ordinarily are considerably more negative than those of aliphatic glucosides. The primary and secondary aliphatic β-*d*-glucosides have molecular rotations falling in the range $-6{,}500$ to $-10{,}500$, while the rotations of the aromatic glucosides, with the exception of the *diortho* substituted derivatives, vary from about $-17{,}000$ to about $-32{,}000$. All of the compounds in table 3 listed above the carboxyphenyl β-*d*-glucosides have "*ortho-para* directing groups" and have molecular rotations between $-17{,}300$ and $-20{,}500$. The remaining substances have "*meta* directing groups" substituted in the benzene nucleus. Seemingly these "*meta* directing groups" when in the *meta* or *para* positions enhance the molecular rotations of the glucosides, and exert their maximum effect when in the *para* position. The effect of the group when in the *ortho* position is usually small. It will be shown in a subsequent section that the effect of the substituent groups on the optical rotations parallels their effect on the dissociation constants of the corresponding phenols.

The molecular rotations of several disubstituted-phenyl glucosides for which one group is an aldehydic radical are also given in table 3. As might be expected from the *meta* directing effect of the CHO group, these compounds have molecular rotations larger than those of aromatic glycosides in which the CHO group is absent. In this

series, however, another factor appears to influence the optical rotation, because the rotation of the compound with the CHO group in the *meta* position is greater than that of the compound in which the CHO group is in the *para* position.

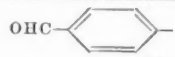
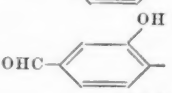
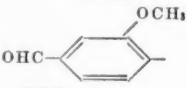
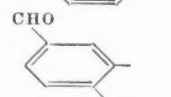
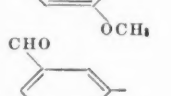
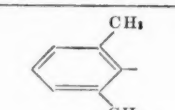
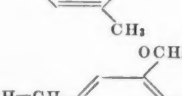
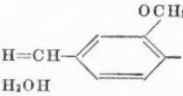
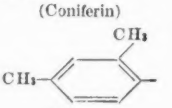
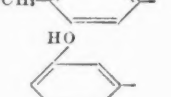
TABLE 2.—*Molecular rotations of aliphatic β-d-glucosides*

Aglucon group	$[M]_D$ in water t=approx. 20° C	$[\alpha]_D$	References
PRIMARY ALCOHOL SERIES			
CH_3-	−6,640	−34.2	56
CH_3-CH_2-	−7,640	−36.7	22, 68
CH_2OH-CH_2-	−6,860	−30.6	69
$CH_3-CH_2-CH_2-$	−8,690	−38.7	68
$CH_2OH-CH_2-CH_2-$	−8,620	−36.2	34
$CH_3-(CH_2)_3-$	−8,720	−36.9	35, 68
$CH_2OH-(CH_2)_3-$	−8,830	−35	34
$CH_3-(CH_2)_5-$	[a] −8,910	−33.7	36
$CH_3-(CH_2)_7-$	[a] −8,860	−30.3	36
$CH_3-(CH_2)_8-$	[a] −8,820	−28.8	36
$CH_3-(CH_2)_9-$	[a] −8,910	−27.8	36
$CH_3-(CH_2)_{11}-$	[a] −8,610	−24.7	36
$CH_3-(CH_2)_{15}-$	[c] −8,900	−22.0	66
ISO SERIES			
$(CH_3)_2CH-$	−8,070	−36.3	68
$(CH_3)_2CH-CH_2-$	−9,430	−39.9	35, 68
$(CH_3)_2CH-CH_2-CH_2-$	−9,110	−36.4	68
TERTIARY ALCOHOL SERIES			
$(CH_3)_3C-$	−4,490	−19.0	35
$(CH_3)_2C(C_2H_5)-$	−4,480	−17.9	35
$CH_3C(C_2H_5)_2-$	−4,490	−17.0	35
$(C_2H_5)_3C-$	−3,730	[b] −13.4	35
CYCLOHEXYL AND BENZYL HOMOLOGOUS SERIES			
$C_6H_{11}-$	−10,860	−41.4	70, 23
$C_6H_{11}-CH_2-$	−10,220	−37.0	23
$C_6H_5-(CH_2)_2-$	−8,140	−27.3	23
$C_6H_5-(CH_2)_3-$	−8,670	−30.5	23
$C_6H_5-CH_2-$	−14,460	−53.5	66, 23
C_6H_5-	−18,190	−71	6, 23
m-$CH_2OH-C_6H_4-CH_2-$	−14,080	−46.9	69
p-$CH_2OH-C_6H_4-CH_2-$	−15,170	−50.5	69
SUBSTITUTED ALCOHOL SERIES			
$CH_2=CH-CH_2-$	−9,290	−42.2	68
$COOH-CH_2-$	−10,500	−44.1	66
$NH_2-CO-CH_2-$	−10,250	−43.2	66
$(CH_2OH)_2CH-$	−7,650	−30.1	74
$CH_3(OCH_3)-CH_2$	−6,840	−28.7	34, 86
$CH_3(OC_2H_5)-CH_2-$	−6,460	−25.6	85
$CH_2(Cl)-CH_2-$	−7,090	−29.2	57
$CH_2(Br)-CH_2-$	−7,490	−26.1	57
$CH_2(I)-CH_2-$	−8,450	−25.3	57
$CH_2(SO_3H)-CH_2-$	−9,480	−32.9	57
$CH_2(SO_3C_2H_5)-CH_2-$	−7,620	−24.1	57
$NH_2-CO-NH-N=CH-CH_2-$	−8,880	−31.8	84
$CH_3OH-CH_2-O-CH_2-CH_2-$	−6,010	−22.4	86

[a] Rotations measured in CH_3OH; t=25° C.
[b] For monohydrate. [c] Rotation measured in C_2H_5OH at 24° C.

TABLE 3.—*Molecular rotations of substituted-phenyl β-d-glucosides*

Substituent group	[M]D when in o-position	[M]D when in m-position	[M]D when in p-position
Mono-Substituted-Phenyl β-Glucosides			
None (H)	(−18, 190)		
OH	−19, 330	−19, 170	−17, 290
OCH_3	−19, 260		
NH_2			−17, 630
CH_3	−18, 570	−18, 840	−18, 300
C_2H_5	−18, 480		−18, 590
CH_2-NH_2	[b]−18, 320	[b]−19, 540	[b]−20, 000
$CH_2-NH-CO-CH_3$	[c]−10, 900	−20, 490	−17, 610
CH_2-COOH			−19, 800
$CH_2-CO-OCH_3$			−19, 040
COOH	−18, 430	−20, 540	−24, 440
$CO-OCH_3$	−20, 240	−23, 290	−24, 550
NO_2	−26, 500	−27, 140	−31, 030
CH_2CN	−19, 670		−21, 080
$CO-CH_3$	−19, 840		−28, 120
CHO	−17, 990		−26, 860

Aglucon group	[M]D
β-Glucosides of Substituted-Aldehydic-Phenols	
	−26, 860
	−27, 920
	−27, 850
	−34, 070
	−29, 550
β-Glucosides of Diorthosubstituted Phenols and Related Compounds	
	−4, 380
	−5, 890
(Syringin)	
	−24, 000
(Coniferin)	
	−18, 480
	−21, 560

[a] Molecular rotations ($[\alpha]_D \times$ mol. wt.) for aqueous solutions at approximately 20° C. For sources of data see table 6, p. 21.

[b] In aqueous acetic acid solution with acid concentration equivalent to the glucoside concentration.

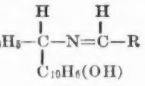
[c] For the *meta* and *para* derivatives, the specific rotation of the acetylated derivatives are recorded as approximately 30° less than those of the deacetylated derivatives, but the reverse is true for the *ortho* compounds. This suggests that one of the values may be in error.

The molecular rotations of *diortho*substituted-phenyl glucosides, given in table 3, show an unexpected trend. The *o,o'*-xylenyl *β-d*-glucoside has a molecular rotation (−4,380) which is about the same value as those characteristic of glycosides derived from tertiary alcohols. In contrast, the isomeric *o,p*-xylenyl *β-d*-glucoside has a value (−18,480) which does not differ widely from what might be expected from the rotations of the monosubstituted phenyl glycosides containing an OH group in either the *ortho* or the *para* position. Although the optical rotation of the *o,o'*- xylenyl *β-d*-glucoside seems unusual, it is probably correct because the analogous compound, syringin, which has two methoxy groups in the *ortho* positions, has a molecular rotation of −5,890 while coniferin, in which only one of the *ortho* positions is substituted, has a molecular rotation of −24,000. The study of other *diortho*substituted derivatives is necessary to show whether or not this property is characteristic of all *diortho*-substituted-phenyl glucosides.

It is interesting to note the resemblance in rotation of the tertiary aliphatic *β*-glucosides and the *diortho*substituted *β*-glucosides. Could this be due to the lack of a hydrogen atom on the carbons near the asymmetric center principally affected? The tertiary aliphatic glucosides and the phenyl glucosides have no hydrogen on the carbon forming the glucosidic linkage. The phenyl glucosides however, have hydrogens in the *ortho* as well as the *meta* positions, which because of the coujugated system present would be expected to act as a hydrogen on the carbon forming the glucosidic linkage. For the *monoortho*substituted-phenyl glucosides one hydrogen atom is replaced and for the disubstituted-phenyl glucosides both hydrogen atoms are replaced by other groups. In respect to hydrogen atoms near the carbon forming the glucosidic linkage, there is then some resemblance between the tertiary aliphatic glucosides and the *diortho*-substituted-phenyl glucosides.

IV. CORRELATION OF THE OPTICAL ROTATION OF AROMATIC GLYCOSIDES WITH THE DISSOCIATION CONSTANTS OF THE CORRESPONDING PHENOLS

In 1930 Betti [7] pointed out a correlation between the optical rotation of substances of the general formula



(where R is a substituted benzene ring) and the dissociation constants of the corresponding acids of the general formula RCOOH. In order to test the possibility that a similar relation might hold for the optical rotations of the substituted phenyl glucosides and the dissociation constants of the corresponding phenols, the optical rotation of phenyl *β-d*-glucoside and of 10 substituted-phenyl *β-d*-glucosides were plotted (see fig. 2 and table 4) against the pK values of the corresponding phenols recently reported by Schwarzenbach and Rudin [8]. As illustrated by figure 2, the points fall fairly close to a straight line. Although the deviations from linearity appear to be greater than the errors of the measurements, it seems that there is an approximate

relationship between the optical rotation and the *pK* values. This correlation is based on 10 points, including six *para*substituted phenols, two *meta*substituted phenols, and one *ortho*substituted phenol. An additional point representing the *o*-nitrophenyl glucoside does not agree with the above correlation but an explanation for this may be found in the well-known tendency of certain *ortho*substituted phenols to form chelate rings by hydrogen bonding, a process which does not seem possible for the corresponding phenyl glucosides.

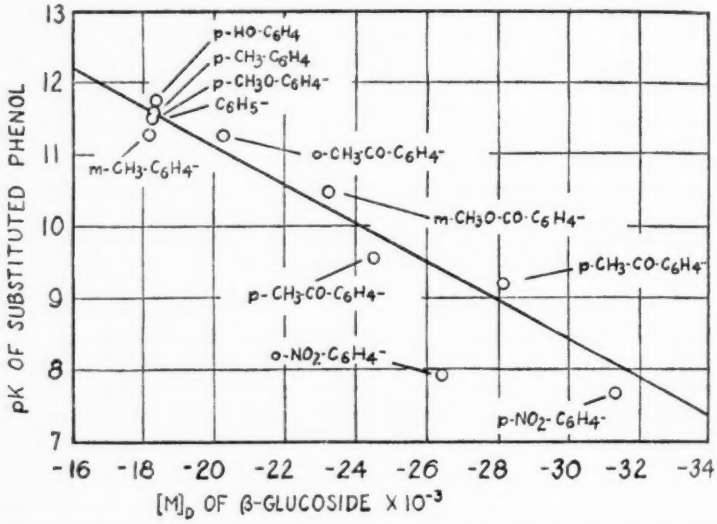


FIGURE 2.—*Relationship between the pK values of phenols and the molecular rotations of the corresponding β-glucosides.*

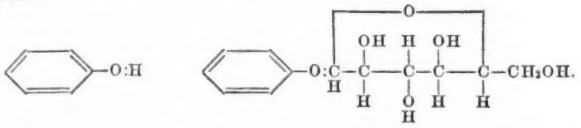
TABLE 4.—*Acid strengths of phenols and molecular rotation of corresponding β-d-glucosides*

Group substituted in the phenol and phenyl glucoside	pK[a]	[b]$[M]_D^{30}$	Group substituted in the phenol and phenyl glucoside	pK[a]	[b]$[M]_D^{30}$
None	11.28	−18,190	*m*-CO-OCH$_3$	10.50	−23,290
p-OH	11.78	−18,390	*p*-CO-OCH$_3$	9.58	−24,550
p-CH$_3$	11.61	−18,300	*p*-CO-CH$_3$	9.21	−28,120
p-OCH$_3$	11.52	−18,240	*p*-NO$_2$	7.68	−31,030
m-CH$_3$	11.43	−18,840	*o*-NO$_2$	7.93	−26,500
o-CO-OCH$_3$	11.28	−20,240			

[a] Data of Schwarzenbach and Rudin [8] for solutions of phenols in alcohol-water mixtures (48 percent of ethyl alcohol) at 20 to 22° C.
[b] Molecular rotation in water. For source of data, see references in table 6.

The linear relationship indicated by figure 2 appears to be in harmony with the observations of Rule [9] and with the calculations of Kauzmann, Walter, and Eyring [10, p. 394]. The compounds used by Betti have the advantage over the glucosides in that they contain only one asymmetric atom, but this advantage is largely offset by the application of Hudson's isorotation principle, which gives at least an approximate value for the optical rotation of the asymmetric center

principally affected. This was shown by the data presented in figure 1, which revealed that the molecular rotation of the aromatic β-glucosides varies approximately directly with the rotatory contribution of the first carbon. The relationship between the optical rotations of the β-glucosides and the dissociation constants is then essentially one between the rotatory contribution of the first carbon and the strength of the bond connecting the first carbon atom and the oxygen atom of the glucosidic linkage. The following formulas illustrate this relation:



For phenols the dissociation constants (also the *pK* values), for constant external conditions, are functions of the strengths of the bonds between the oxygen atoms and the ionizable hydrogen atoms. In the phenyl glucosides this bond, as represented above, is the bond connecting the oxygen atom to the asymmetric carbon atom. Thus, the pair of electrons forming this bond appears to play an important part in determining the magnitude of the optical rotation.

The relationship between the rotatory power of the aromatic β-glucosides and the dissociation constants of the phenols may be of value for estimating approximate *pK* values for the phenols. As mentioned previously, however, *ortho*substituted phenols and their glucosides may provide an exceptional class because of the proximity of the group in the *ortho* position to the phenolic oxygen. This may be particularly evident when the group in the *ortho* position readily forms chelate rings. Inasmuch as the dissociation constants of the phenols are considerably larger than the dissociation constants of the alcohols and of water, the smaller levorotations of the aliphatic β-*d*-glucosides and of β-*d*-glucose appear to be in qualitative accord with a correlation of the optical rotation with the dissociation constants.

TABLE 5.—*Specific and molecular rotations of α- and β-glycosides*

Substance	Not acetylated				Acetylated				References
	α-Isomer		β-Isomer		α-Isomer		β-Isomer		
	[a]D (H2O)	[M]D	[a]D (H2O)	[M]D	[a]D (CHCl3)	[M]D	[a]D (CHCl3)	[M]D	
l-Arabinose	[b]+34.7	+11,560	+190.6	+28,610	+42.5	+13,500	+147.2	+46,800	15, 45, 46
Methyl *l*-arabinoside	+17.3	+2,840	+245.5	+40,300					47
Ethyl *l*-arabinoside	+14.4	+2,570	+233.5	+41,610					16
Phenyl *l*-arabinoside	+6.0	+1,360	+243	+54,970					17, 33
Phenyl *d*-arabinoside	−5.5	−1,240	+244	+55,200					33
d-Galactose	+150.7	+27,150	+52.8	+9,510	+107	+41,770	+23	+8,980	15, 48, 49
Methyl *d*-galactoside	+196.1	+38,080	0.7	+140	+133.0	+48,200	−14.0	−5,070	44
Phenyl *d*-galactoside	+217	+55,610	−39.8	−10,200	+175.5	+74,480	−0.7	−300	6, 16, 72, 73
o-Tolyl *d*-galactoside	+188	+50,810	−43.3	−11,700	+173	+75,840	−4	−1,750	6, 18, 19
d-*a*-Glucoheptose					+87.0	+40,230	+4.8	+2,220	50, 51, 52
Methyl *d*-*a*-glucoheptoside	+111.5	+25,000	−74.9	−16,790	+107.4	+46,650	−16	−6,950	20, 53, 54
Phenyl *d*-*a*-glucoheptoside	+163	+46,660	−89.7	−25,680	+167	+82,900	+8.0	+3,970	21
d-Glucose	+112.2	+20,210	+18.7	+3,370	+101.6	+39,700	+3.8	+1,480	15, 55, 56, 62
Methyl *d*-glucoside	+158.9	+30,860	−34.2	−6,640	+130.5	+47,280	−18.2	−6,590	56, 55, 62
Ethyl *d*-glucoside	+152.0	+31,650	−36.7	−7,640	+132.1	+49,710	−22.7	−8,540	22, 67, 68
Propyl *d*-glucoside	+140.8	+31,290	−38.7	−8,600					67, 68
Ethylene glycol *d*-glucoside	+135.5	+30,380	−30.6	−6,860					69
n-Hexyl *d*-glucoside					+116.6	+50,240	−19.9	−8,610	70
Cyclohexyl *d*-glucoside	+133.2	+34,940	−41.4	−10,860	+121.7	+52,380	−29.4	−12,650	70, 66, 23
Benzyl *d*-glucoside	+131	+35,410	−53.5	−14,460	+143.3	+62,820	−52.3	−22,930	23, 65, 66
Phenyl *d*-glucoside	[c]+180.8	+49,590	−71	−18,190			−22.0	−9,340	6, 23
p-Nitrophenyl *d*-glucoside	+215	+64,770	−103	−31,030	+124	+71,300	−41	−19,240	24, 25, 26
Allyl *d*-glucoside	+131.7	+29,000	−42.2	−9,290					67, 68
1,3-Propanediol *d*-glucoside	+137.5	+32,760	−36.2	−8,620					34, 76
p-Aminophenyl *d*-glucoside	[d]+194.1	[d]52,650	−65	−17,630					25, 26
p-Hydroxyphenyl *d*-glucoside	+178.6	+48,620	−63.5	−17,290					28, 71
p-Benzoylphenyl *d*-glucoside					+137.7	+74,970	−14.5	−7,890	28
d-*a*-Mannoheptose	[c]+120.0	+27,380	[c]+42.3	+9,650	+120.8	+55,860	+34.1	+15,770	13, 29
Phenyl *d*-*a*-Mannoheptoside	+207	+59,260	−39.8	−11,390					21
d-Mannose	+29.3	+5,280	−17.0	−3,060	+55.0	+21,470	−25.2	−9,840	15, 58, 59
Methyl *d*-mannoside	+79.2	+15,380	[c]−53.3	−13,550	+49.1	+17,790	−50.4	−18,260	20, 60, 61, 62
Phenyl *d*-mannoside	+113.5	+29,080	−71.6	−18,350	+73.9	+31,360	−62.7	−26,610	30
l-Rhamnose	−8.6	−1,570	+38.4	+6,300					15, 31
Methyl *l*-rhamnoside	−62.1	−11,140	+95.4	+17,000	[f]−53.7	[f]−16,340	[f]+45.7	[f]+13,910	63, 62
Phenyl *l*-rhamnoside	−106	−25,470	+87.5	+21,020	−80	−29,310	+52.5	+19,230	33

[a] Measured at approximately 20° C.
[b] For *l*-arabinose-$CaCl_2$-$4H_2O$.
[c] For monohydrate.
[d] In CH_3OH solution.
[e] For β-methyl mannoside-$(CH_3)_2$—CHOH.
[f] In acetylene tetrachloride solution.

TABLE 6.—*Specific rotation of substituted-phenyl β-d-glucosides*

Substituent group	$^a[\alpha]_D$ When in *o*-position	When in *m*-position	When in *p*-position	References	Substituent groups	$^a[\alpha]_D$	References
MONOSUBSTITUTED PHENYL β-GLUCOSIDES					β-glucosides of substituted-aldehydic-phenols		
H (none)	(−71)			6, 23	4-CHO, 2-OH	−93	18
OH	−71	−70.4	−63.5	37, 71	4-CHO, 2-OCH_3	−88.6	80
OCH_3	[b]−66.8			38	3-CHO, 6-OCH_3	−108.4	18
NH_2			−65	26	3-CHO, 6-OC_2H_5	−90	18
CH_3	−68.7	−69.7	−67.7	19, 39			
C_2H_5	−65		−65.4	18	β-glucosides of diorthosubstituted-phenols and related compounds		
CH_2-NH_2	[c]−64.2	[c]−68.5	[c]−70.1	39.40			
$CH_2-NH-CO-CH_3$	[d]−33.3	−62.6	−53.8	39.40			
CH_2-COOH			−63	18			
$CH_2-CO-OCH_3$			−58	18			
COOH	[e]−59.6	−68.4	−81.4	41.77	2-CH_3, 6-CH_3	−15.4	19
$CO-OCH_3$	−64.4	−74.1	−78.1	41, 78	2-OCH_3, 4-(CH_2OH-CH=CH-), 6-OCH_3	−17.3	81
NO_2	[b]−83	[b]−85	−103	24, 26, 42	4-(CH_2OH-CH=CH-), 6-OCH_3	−70.1	82
CH_2CN	−66.6		−71.4	18	2-CH_3, 4-CH_3	−65	18
$CO-CH_3$	−66.5		[b]−88.9	18, 77	3-OH, 5-OH	−74.8	75
CHO	[f]−60.4		−94.5	43, 79			

[a] In water at approximately 20° C.
[b] For the monohydrate.
[c] For aqueous solutions containing acetic acid equivalent to the glucoside concentration.
[d] See table 3, footnote c.
[e] For glucoside-1/2 H_2O.
[f] For glucoside-3/4 H_2O.

V. EXPERIMENTAL DETAILS

1. GENERAL PROCEDURE

The new phenyl glycosides were prepared by the method of Helferich and Schmitz-Hillebrecht [6]. The method consists in heating an acetylated sugar with phenol and a catalyst (anhydrous zinc chloride or *p*-toluenesulphonic acid). After the mixture has cooled, it is extracted with benzene and water and the benzene extracts are washed with 2 *N* sodium hydroxide to remove the excess phenol. The benzene extracts upon evaporation give the acetylated glycoside, which upon solution in absolute methanol and treatment with very small amounts of barium methylate at the boiling point for 10 minutes [11, 12] is converted to the phenyl glycoside.

For each of the following compounds, the time of heating and the catalyst used are given.

2. PHENYL *d*-α-MANNOHEPTOSIDES

Preparation of Hexaacetyl-β-d-α-mannoheptose.

The preparation of this compound was previously reported by Montgomery and Hudson [13], but as considerably better yields have been obtained, the method used is described.

To a mixture of 50 ml of acetic anhydride and 70 ml of pyridine at room temperature there was added in small portions 10 g of *d*-α-mannoheptose. The solution was kept at room temperature for 48 hours and then poured into ice water. Crystallization occurred, the crystals were separated by filtration, and the filtrate was extracted with chloroform. A total of 18.5 g of crude hexacetyl-β-*d*-α-manno-

heptose was obtained (17 g by direct crystallization and 1.5 g from the chloroform extract). This corresponds to a yield of 80 percent of the theory as compared to one of 58 percent reported by Montgomery and Hudson [13] by the sodium acetate method.

Phenyl d-α-mannoheptosides.

Under the condition employed, both catalysts (zinc chloride and *p*-toluenesulphonic acid) gave mixtures of the α- and β-glycosides and the acetylated glycosides were not obtained in the crystalline condition.

A mixture of 3 g of hexaacetyl-β-*d*-α-mannoheptose, 3 g of phenol, and 0.2 g of *p*-toluenesulphonic acid was heated on the boiling water bath for 1 hour, and the mixture was treated as described under the "general procedure" (p. 21). A sirup was obtained which upon deacetylation gave the crystalline phenyl *d*-α-mannoheptosides. The first material obtained from methyl alcohol was fairly pure alpha isomer, while the mother liquors gave mixtures of the two isomers. About 0.3 g of the alpha and 0.3 g of the mixture of isomers were obtained. These were purified as described below.

The heating of a mixture of 3 g of the hexaacetyl-β-*d*-α-mannoheptose, 3 g of phenol, and 1 g of anhydrous zinc chloride for 1 hour on the boiling water bath gave practically the same proportions and amounts of the two isomers as when the previous procedure was used.

The impure alpha isomer (mp 195° to 205° C) obtained from dilute methyl alcohol solutions of the deacetylated mixture is readily purified by recrystallization from methyl alcohol. The pure material melts at 212° C (corr.), and the rotation in water is $[\alpha]_D^{20}=+207$ (*c*, 0.8; 0.0789 g made up to a volume of 10 ml read 9.45° S in a 2-dm tube). The alpha isomer is the less soluble of the two isomers in water and in methyl alcohol, and a saturated aqueous solution at 20° C contains about 0.8 g in 100 ml. Analysis: Calculated for $C_{13}H_{18}O_7$: C, 54.54; H, 6.34; Found: C, 54.8; H, 6.3.

Considerable difficulty was experienced in the preparation of the pure beta isomer, but the following procedure was found to be satisfactory. One gram of a mixture was dissolved in 6 ml of hot water, and the solution was cooled. The crystals which separated were fairly pure alpha isomer. The solution remaining was evaporated to dryness and the residue recrystallized from 25 ml of hot absolute alcohol. The material so obtained had a rotation of $[\alpha]_D^{20}=-23$. This mixture after two recrystallizations from absolute alcohol gave pure phenyl β-*d*-α-mannoheptoside with melting point of 189 to 190° C (corr.) and $[\alpha]_D^{20}=-39.8$ (water; *c*, 1.1; 0.1100 g made up to a volume of 10 ml read −2.53° S in a 2-dm tube). Analysis: Calculated for $C_{13}H_{18}O_7$: C, 54.54; H, 6.34; Found: C, 54.2; H, 6.0.

3. PHENYL *d*-TALOSIDES

A mixture of 2.5 g of pentaacetyl-α-*d*-talose, 2.5 g of phenol, and 0.5 g of anhydrous zinc chloride was heated on the water bath for 2 hours. The product was carried through the general procedure described above and gave a sirup which crystallized from ethyl alcohol. Yield: 1.0 g of phenyl tetraacetyl-α-*d*-taloside. This was purified by several recrystallizations from methyl or ethyl alcohol. The purified material melted at 103.5 to 104.0° C (corr.). The rotation was $[\alpha]_D^{20}=+97.4$ ($CHCl_3$; *c*, 3.5; 0.1052 g made up to a volume of 3.024

ml read +9.79° S in a 1-dm tube). Analysis: Calculated for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70; Found: C, 56.7; H, 5.5.

The phenyl *α-d*-taloside was obtained by deactylating the tetraacetate, and after several recrystallizations from alcohol and finally from water it had a melting point of 165.5 to 166.5° C (corr.) and a rotation of $[\alpha]_D^{20}=+138$ (H_2O; *c*, 0.9; 0.0894 g made up to a volume of 10 ml, read 7.11° S in a 2-dm tube). The solubility of the compound in water at 20° is 0.5 g in 100 ml. Analysis: Calculated for $C_{12}H_{16}O_6$: C, 56.24; H, 6.29; Found: C, 56.3; H, 6.2.

4. PHENYL *d-α*-GLUCOHEPTOSIDES

A mixture of 6.7 g of hexaacetyl-*β-d-α*-glucoheptose, 7 g of phenol, and 2 g of anhydrous zinc chloride was heated for 2 hours in a boiling water bath. The product was treated as previously described and the sirup, when dissolved in ethyl alcohol, crystallized. The first impure crystals, consisting principally of the alpha isomer, which appear under the microscope as square or rectangular plates, melted at 140° C and weighed 2.1 g. The mother liquors yielded about 1 g more of a mixture of the alpha and beta forms (melting point approximately 100° C). Total yield: 3.1 g.

The higher-melting material upon recrystallization from methyl alcohol readily gave the pure phenyl pentaacetyl-*d-α*-glucoheptoside, which melts at 154.0 to 155.0° C (corr.) and gives $[\alpha]_D^{20}=+167$ ($CHCl_3$; *c*, 3.0; 0.3022 g made up to a volume of 10 ml read 29.15° S in a 2-dm tube). Analysis: Calculated for $C_{23}H_{28}O_{12}$: C, 55.64; H, 5.68; Found: C, 55.8; H, 5.8.

The beta isomer, which crystallizes from alcohol in pointed prisms, was more difficult to purify. However, purification was accomplished by crystallization from carbon tetrachloride as described by Fischer for the phenyl galactosides. The relative solubilities of the alpha and beta isomers of the acetylated phenyl glycosides are apparently completely different in carbon tetrachloride from those in solvents such as the alcohols. In 2 ml of warm carbon tetrachloride, there was dissolved 0.4 g of a mixture of the two isomers ($[\alpha]_D^{20}=+38$). After the solution had stood for some time at +5° C, the crystals were separated (wt 0.12 g) and found to have the rotation: $[\alpha]_D^{20}=+9.9$. The phenyl pentaacetyl-*β-d-α*-glucoheptoside obtained by recrystallizing the above material several times from absolute ethyl alcohol melted at 97° C (corr.). The rotation was $[\alpha]_D^{20}=+8.0$ ($CHCl_3$; *c*, 1.1; 0.1107 g made up to a volume of 10 ml read +0.51° S in a 2-dm tube). Analysis: Calculated for $C_{23}H_{28}O_{12}$: C, 55.64; H, 5.69; Found: C, 55.8; H, 5.6.

The phenyl *α-d-α*-glucoheptoside, obtained by deacetylation of the pentaacetate, after recrystallization from methyl alcohol melted at 191 to 192° C (corr.) and had as the specific rotation $[\alpha]_D^{20}=+163$ (H_2O; *c*, 1.0; 0.1015 g made up to a volume of 10 ml read +9.74° S in a 2-dm tube). Analysis: Calculated for $C_{13}H_{18}O_7$: C, 54.54; H, 6.34; Found: C, 54.8; H, 6.3.

The phenyl *β-d-α*-glucoheptoside, obtained in a similar manner, after one recrystallization from isopropyl alcohol melted at 167 to 168° C (corr.). The specific rotation was measured as $[\alpha]_D^{20}=-89.7$ (H_2O; *c*, 0.9; 0.0272 g made up to a volume of 3.024 ml read 2.33° S in a 1-dm tube). Analysis: Calculated for $C_{13}H_{18}O_7$: C, 54.54; H, 6.34; Found: C,54.4; H, 6.3.

VI. REFERENCES

Many of the rotational values used in the present paper are assembled in the several handbooks available (see [4] and [14]. Reference [1] and Circular C429 of the National Bureau of Standards, Polarimetry, Saccharimetry and the Sugars (publication pending) have also very useful compilations of optical rotational values for the sugars and derivatives. The references given in the present paper are to the actual data used and not necessarily to original work which, however, can be located from the above-mentioned compilations.

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RESEARCH PAPER RP1400

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EFFECT OF THE CHEMICAL DURABILITY OF GLASS ON THE ASYMMETRY POTENTIAL AND REVERSIBILITY OF THE GLASS ELECTRODE

By Edgar H. Hamilton and Donald Hubbard

ABSTRACT

Departures from a linear relation between the voltage of the glass electrode and the pH of alkaline solutions were approximately proportional to the amount of attack on the glass by the solution, as measured by the interferometer. The departures were less before than after the electrode had been immersed in a highly alkaline solution, and the drift of the potential at a constant pH was most marked with high alkalinity, indicating that nonreproducibility of voltage was associated with chemical attack on the glass. Differences in the capacities of the two surfaces of electrode bulbs of Corning 015 glass to absorb ions, as shown by the absorption of an organic dye, may be the cause of asymmetry potential.

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I. INTRODUCTION

The glass electrode is subject to voltage departures from the straight-line relation of the Nernst equation in the pH ranges below 2 and above 9 [1],[1] and also in solutions of high salt concentrations [2]. Previous work has demonstrated that these voltage departures are always associated with changes in the chemical durability of the glass from which the electrodes are made [3]. Other conspicuous features of the glass electrode are a gradual potential drift and irreversibility in the alkaline range and an asymmetry potential. That these phenomena might also be associated with the chemical durability of the glass is suggested by this earlier work.

II. EXPERIMENTAL PROCEDURE

The glass used throughout this investigation was Corning 015 [4]. All emf measurements were made at room temperature with a portable

[1] Figures in brackets indicate the literature references at the end of this paper.

potentiometer-electrometer. The pH determinations were obtained by means of a hydrogen electrode, with a saturated calomel half-cell as a reference electrode.

Durability measurements, for studying the relation between voltage departure and chemical durability, were made by the interferometer method [3, 5]. The dye-absorption method with Victoria Blue B, which has been used successfully for rating the relative durability of glass containers of similar composition [6], was applied for differentiating between the inner and outer surfaces of blown bulbs.

III. REVERSIBILITY

All of the electrodes of Corning 015 glass used in this investigation show a certain degree of irreversibility at any given pH in the high-alkaline region in addition to a voltage departure. At pH 12, for example, the voltage departure depends on whether the electrode has been brought from a more acid medium or a more alkaline one. In addition to this, the voltage of the electrode in strongly alkaline solution does not remain constant, but undergoes a voltage drift, the rate of which is partially determined by the alkalinity. Such behavior is to be expected if the glass electrode is undergoing attack by the solution.

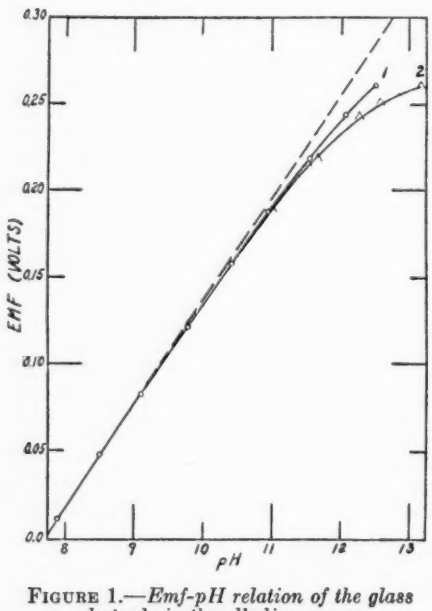

FIGURE 1.—*Emf-pH relation of the glass electrode in the alkaline range.*

(All potentials corrected to 25° C.)
○, Increasing pH obtained by HAc+NaOH.
△, Decreasing pH obtained by NaOH+HAc.

To study this irreversibility, a solution of 0.2 *N* acetic acid was titrated with 0.2 *N* NaOH. At suitable stages of the titration, simultaneous pH determinations of the solution and emf readings of the glass electrode immersed in it were made. The titration was continued up to a pH of 12.5. At the end of this experiment, the same electrode was placed in a sodium hydroxide solution of pH 13.2. This solution was then titrated with acetic acid, and pH and emf readings made as before, until the solution had reached a pH of 11.2.

The data plotted in figure 1 show the pH-emf relation and the voltage departures of the glass electrode in the alkaline range in the presence of Na^+ ions. As the alkalinity is increased to pH 12.5,

accompanied by an increase in Na^+ ion concentration, the departure from the straight-line relation increases; and, conversely, as the alkalinity is decreased from pH 13.2 by the addition of acetic acid, the voltage departure decreases, but in this latter case the voltage departure at a given pH is always the greater. This lack of reversibility no doubt accounts for the greater part of the differences between the voltage departures reported by various investigators [2, 3, 4].

IV. COMPARISON OF VOLTAGE DEPARTURES AND CHEMICAL DURABILITY

1. DATA AND RESULTS

In order to determine what relation exists between the voltage departures and the chemical durability of the electrode glass, durability measurements obtained on Corning 015 glass by the interferometer method were compared with voltage departures reported by MacInnes and Dole [4], and Dole [2], and those, shown in figure 1, obtained by the authors. The departure data were interpolated to the pH values at which durability measurements were made. The durabilities were measured at 80° C, but it has been shown that attack on the glass at any other temperature can be approximated from the equation: Attack $b^{\cdot 2}$ (t/10) [5], in which b is a constant and t is the temperature in degrees centigrade. Such durability values calculated for 25° C are given in the third column of table 1, and plotted against voltage departures in figure 2. Although the electrode readings were taken after a short exposure and durability measurements were for a 6-hour period, a comparison is justifiable, because the amount of attack is directly proportional to the duration of exposure. Figure 2 shows that the relationship between voltage departure and attack is linear within the limits of error for measuring the attack.

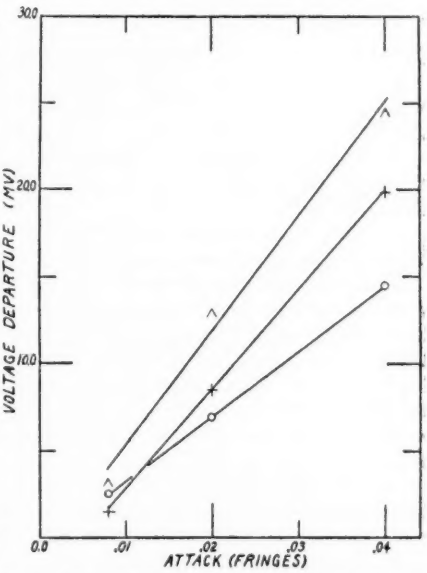

FIGURE 2.—*Relation between voltage departures of the glass electrode and the chemical durability of the electrode glass.*

(Calculated to 25° C). Sources of voltage departure data:
○, Curve 1 of figure 1.
+, D. A. MacInnes and M. Dole.
△, M. Dole.

TABLE 1.—*Voltage departures of the glass electrode and the chemical durability of Corning 015 electrode glass*

pH	Attack in 6 hours at—		Voltage departures at 25° C as reported by—		
	80° C (determined)	25° C (calculated) [a]	Curve 1 figure 1	MacInnes [b] and Dole	Dole [c]
	Fringes	*Fringes*	*mv*	*mv*	*mv*
9.9	0.35	0.008	2.5	1.5	3.0
11.2	.90	.020	7.0	8.6	12.8
12.0	1.75, 1.90	.040	14.5	19.9	24.3

[a] Attack $=b \cdot 2^{(t/10)}$, where b is a constant and t is the temperature in degrees centigrade.
[b] J. Am. Chem. Soc. **52,** 35 (1930), figure 3, curve B.
[c] J. Am. Chem. Soc. **53,** 4266 (1931), figure 2, bottom curve.

2. DISCUSSION OF RESULTS

Any quantitative relation between voltage departures and chemical durability must, of necessity, not be accepted too rigorously, since:

1. The glass is not entirely uniform and contains many cords and striae, which make an estimate of the amount of shift in the interferometer fringes difficult.

2. Voltage departures and chemical durability must be determined on separate specimens, blown bulbs in the first case and cloth-polished flats in the latter.

3. The slight drift in the potential of the glass electrode in the alkaline range raises a question as to its true potential.

The amount of attack on the electrode by the solution is actually very minute. One fringe is equal to approximately 0.29 micron, and the density of the glass is 2.5. From these data, it can be calculated that 1.83 fringes, the attack at pH 12 at 80° C for 6 hours, is equal to 0.133 mg of glass per square centimeter of surface. The rate of attack would be much slower at 25° C, at which temperature the electrode measurements were made. A calculation based on the effect of temperature on the chemical durability of Corning 015 glass [5] gives 0.04 fringe, or 0.0029 mg of glass per square centimeter, as the amount of attack by the above solution in 6 hours at 25° C. This very low solution rate may account for the slowness of the potential drift of the glass electrode [4].

V. DIFFERENCES IN INNER AND OUTER SURFACES OF ELECTRODE BULBS

1. MEASUREMENT OF DYE ABSORPTION

Since the voltage anomalies of the glass electrode have been definitely associated with changes in the chemical durability of the glass from which these electrodes are made, it is logical to look to chemical durability for an explanation of the asymmetry potential of the glass electrode. It has been demonstrated that in the pH range 2 to 7 the electrode glass exhibits a slight uniform swelling [3]. If it can also be demonstrated that the two surfaces of a glass electrode are different, as, for instance, in their abilities to swell or to absorb ions from a solution, this difference might account for the "asymmetry potential"—that is, the potential which is obtained when the inner and outer surfaces of the electrode bulb are in contact with the same solution.

We have previously observed that the outer surface of a glass bottle is much more durable than its inner surface, unless the latter has been especially treated. To determine if the same condition exists in electrode bulbs, large bulbs (50- to 100-ml capacity) of Corning 015 glass were blown on the end of soft glass tubes (15-mm inside diameter). The bulbs were filled with a pH 6 sodium-potassium phthalate buffer and suspended in a Pyrex beaker containing the same buffer solution [6, 7]. The assembly was placed in a thermostated water bath at 80° C for 24 hours. At the end of this period the buffer was removed and the bulbs were rinsed thoroughly with hot distilled water. Both surfaces of the bulbs were dyed at room temperature for 30 minutes in a 0.06-percent aqueous solution of Victoria Blue B (a basic dye) containing 1 ml of acetic acid per 100 ml of solution. All excess dye was removed by rinsing 3 to 4 times with distilled water, followed by two 10-minute extractions with distilled water. All dye on the surfaces of the soft glass tubing was removed by swabbing with alcohol-saturated cotton wads. The dye absorbed on the surfaces of the bulbs was extracted in known volumes of 95-percent ethanol. The amount of dye was determined by measuring the light absorbed by the alcoholic solutions with the aid of a photoelectric cell, using an incandescent lamp as a light source and a light filter composed of Corning glasses G34Y and G555Q. From table 2, it may be seen that the amount of dye absorbed by the inner surfaces was from 19 to 36 times that absorbed by the outer surfaces of the respective bulbs.

TABLE 2.—*Comparison of chemical durability of inner and outer surfaces of bulbs of Corning 015 glass as indicated by absorption of Victoria Blue B dye*

Bulb	Dye absorbed by—		Ratio *I/O*
	Inner surface, *I*	Outer surface, *O*	
	mg	*mg*	
A	0.355	0.019	18.7
B	.407	.016	25.5
C	.310	.015	20.7
D	.535	.015	35.6

This difference between the two surfaces is due to the treatment o the glass during the blowing process. The outer surface was formed from glass in or near the outer surface of the button. During the heating of the glass to its working temperature, the volatile constituents in the surface of the button are continuously being removed by the stream of hot gases from the flame. The glass which forms the inner surface of the bulb is not exposed to this stream of hot gases, and therefore its composition is not altered, or at least not to such an extent as the glass in the outer surface.

2. POSSIBLE RELATIONSHIP TO ASYMMETRY POTENTIAL

When a glass electrode bulb is filled with and placed in water, or an aqueous solution of pH 2 to 7, both surfaces will absorb ions and swelling will take place. Since the inner surface is capable of absorbing a different quantity of ions than the outer surface, as indicated by the Victoria Blue *B* test, the inner surface will assume a charge of dif-

ferent magnitude than the outer surface. Such a condition will give rise to a potential difference between the two surfaces and is probably the source of the asymmetry potential.

3. EFFECT OF ALTERATION OF OUTER SURFACE

Any change in the solution in which the electrode is immersed which affects the chemical nature of the outer surface will necessarily affect its absorbing ability and hence the voltage at this surface. Such is the case in the alkaline range, in which the more durable silica layer is dissolved. The magnitude of this change in the surface can be qualitatively visualized by noting that the ability to absorb Victoria Blue *B* dye is completely eliminated when the glass surface has been exposed to NaOH. Since the inner surface of the electrode bulb is always in contact with the same solution, the changes in the outer surface are probably the largest sources of voltage departures of the glass electrode.

VI. CONCLUSIONS

The outer surface of the bulb of a glass electrode is attacked appreciably when immersed in alkaline solutions of pH greater than about 9. Since a marked voltage departure, proportional to the rate of attack, occurs in this region, it is reasonable to consider that the voltage departure is a result of the attack and depends on the chemical durability of the glass. A simple and satisfactory explanation of the voltage departure is thus obtained.

The conclusion is drawn that the asymmetry potential of the glass electrode results from differences in the ability of the inner and outer surfaces of the electrode to absorb ions.

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AN IMPROVEMENT IN THE "PARTITION METHOD" FOR THE DETERMINATION OF BORON

By Francis W. Glaze and Alfred N. Finn

ABSTRACT

In a previous paper a method was described for the routine determination of boron in glass, based on the "partition" of boric acid between water and ether in the presence of hydrochloric acid and ethanol. In that method, barium, fluorine, iron, and zinc interfere. It has now been found that the substitution of sulfuric acid for hydrochloric acid eliminates the interference of all of these elements except fluorine and gives results no less satisfactory than the previous method. Accordingly, the procedure reported in this paper should be substituted for the one originally described when one has occasion to determine boron in a glass of unknown composition or when the interfering elements are present.

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I. INTRODUCTION

An earlier publication [1] reported a method for the determination of boron in glass which depended on the "partition" of boric acid between the acidified water extract of the sodium carbonate fusion of the sample and an ether-ethanol mixture. Hydrochloric acid was used to acidify the water extract. It was found that barium, fluorine, iron, and zinc interfered. Inasmuch as sulfates are as a rule insoluble in ethanol, it was decided to substitute sulfuric acid for hydrochloric acid, hoping thereby to counteract the above interferences of barium, iron, and zinc.

II. EXPERIMENTAL STUDY

As hydrochloric acid (sp gr 1.18) is approximately 11.6 *N*, sulfuric acid of that normality [2] was used as a starting point; the above acid, diluted with an equal volume of distilled water, was used in place of the hydrochloric acid (1:1).

After several preliminary experiments showed promise, it was decided to determine the temperature effect before proceeding further. One-gram samples of sodium carbonate and measured volumes of a standard boric acid solution were added to calibrated, glass-

[1] Francis W. Glaze and A. N. Finn, J. Research NBS **16**, 421 (1936) RP882.
[2] 32.3 ml of sulfuric acid (sp gr 1.84) made up to 100 ml.

stoppered graduates, made just acid to *p*-nitrophenol with sulfuric acid (approximately 5.8 *N*); and then 1 ml of sulfuric acid (approximately 11.6 *N*) was added. The volumes were adjusted to 25 ml with distilled water, and then 25 ml of absolute ethanol and 50 ml of ether were added. The graduates were shaken at various temperatures between 20° and 30° C, the layers allowed to separate, and 50-ml aliquot portions of the ether-ethanol layers taken. The boron in each was determined, and the "partition coefficients" were calculated from the boron concentrations in the two layers. On plotting the coefficients, k, against the temperature, it was found that, in the range 20° to 30° C, the relation is linear and can be represented by the equation

$$k=0.417-0.00232t.$$

In studying the effect of the various constituents under consideration, Standard Sample 93 (glass, high boron) of the National Bureau of Standards was used in most cases as the source of boron. The sample, plus the constituent being tested, weighed, in all cases, 0.5000 g.

TABLE 1.—*Results obtained for B_2O_3 in the presence of varying amounts of zinc, barium, iron, aluminum, and lithium*

Experiment number	B_2O_3 present	Constituent being tested		Extraction temperature	Et_2O layer	H_2O layer	k[1] (calculated)	B_2O_3[2] present	B_2O_3[2] found	Difference
	g		*g*	*°C*	*ml*	*ml*		*%*	*%*	*%*
1	0.06300	ZnO	None	24.0	76.5	21.9	0.361	12.60	12.52	−0.08
2	.06174	do	0.01	24.0	76.5	22.0	.361	12.35	12.43	+.08
3	.06048	do	.02	24.0	76.8	21.7	.361	12.10	11.99	−.11
4	.05922	do	.03	22.5	76.5	22.1	.365	11.84	11.76	−.08
5	.05796	do	.04	25.0	77.0	21.6	.359	11.59	11.46	−.13
6	.05670	do	.05	25.0	76.9	21.7	.359	11.34	11.21	−.13
7	.06300	BaO	None	25.0	76.7	21.6	.359	12.60	12.55	−.05
8	.05670	do	.05	25.0	76.6	21.8	.359	11.34	11.17	−.17
9	.05040	do	.10	24.5	76.8	21.7	.360	10.08	10.09	+.01
10	.04410	do	.15	25.0	76.7	21.7	.359	8.82	8.82	±.00
11	.03780	do	.20	25.3	76.7	21.8	.358	7.56	7.47	−.09
12	.06237	Fe_2O_3	.005	25.3	76.9	21.3	.358	12.47	12.39	−.08
13	.05985	do	.025	25.3	76.8	21.5	.358	11.97	11.99	+.02
14	.05670	do	.05	26.0	76.8	21.5	.357	11.34	11.43	+.09
15	.06880	Al_2O_3	.025	25.0	77.7	20.8	.359	13.76	13.71	−.05
16	.06880	do BaO	.025 .05	25.0	77.4	21.1	.359	13.76	13.77	+.01
17	.06880	Al_2O_3 BaO	.025 .15	25.3	77.5	20.8	.358	13.76	13.71	−.05
18	.06880	Al_2O_3 BaO	.025 .20	25.0	77.0	21.5	.359	13.76	13.67	−.09
19	.06880	Al_2O_3	.05	24.5	77.9	20.5	.360	13.76	13.74	−.02
20	.06880	do BaO	.05 .05	25.0	78.2	20.0	.359	13.76	13.77	+.01
21	.06880	Al_2O_3 BaO	.05 .10	23.0	77.9	20.2	.364	13.76	13.76	±.00
22	.06880	Al_2O_3 BaO	.05 .15	23.5	77.9	20.7	.362	13.76	13.67	−.09
23	.06880	Al_2O_3 BaO	.05 .20	25.0	77.9	20.5	.359	13.76	13.81	+.05
24	.00684	Li_2O	.05	26.0	78.3	20.7	.357	1.37	1.32	−.05
25	.05650	do	.05	26.0	78.7	20.3	.357	11.30	11.25	−.05

[1] $k=0.417-0.00232t$.

[2] On the assumption that the "B_2O_3 present" was in a 0.5000-g sample of glass.

[3] Percentage of $B_2O_3=4(B_2O_3)_{50\ Et_2O}\left[V_{Et_2O}+\frac{V_{H_2O}}{k}\right]$, where V_{Et_2O} and V_{H_2O} are the volumes of the ether-ethanol layer and the water layer, respectively, and $(B_2O_3)_{50\ Et_2O}$ represents the grams of B_2O_3 in the 50-ml aliquot sample of the ether-ethanol layer.

In the case of zinc, mixtures of zinc oxide and the sample were fused in a platinum crucible with 1 g of sodium carbonate, cooled, and neutralized with sulfuric acid (approximately 5.8 *N*) to the *p*-nitrophenol end point. The boron in the resulting mixture (which contained some precipitated silica, etc.) was then determined. From table 1, experiments 1 to 6, it can be seen that, though there is a tendency for the results to be slightly low, they are reasonably satisfactory in the presence of as much as 10 percent of zinc oxide.

Because of its extensive use in optical glasses, barium oxide in amounts as high as 40 percent was added. As can be seen from experiments 7 to 11, table 1, the results are very satisfactory, except for experiment 8.

Although the very small amounts of iron ordinarily present in glass did not interfere in the original method, yet amounts equivalent to 1 percent of ferric oxide colored the ether-ethanol layer and masked the *p*-nitrophenol end point. However, amounts up to 10 percent do not interfere in the present modification, as can be seen from experiments 12 to 14 in table 1.

In experiments 15 to 23, NBS Standard Sample 80 (glass, soda-lime) (no boron) was used as the glass and a standardized boric acid solution was added to the cooled melt. These tests show that alumina up to 10 percent in combination with barium oxide up to 40 percent does not interfere.

In the last two experiments, lithium equivalent to 10 percent of lithium oxide in the sample was added as the carbonate to NBS Standard Samples Nos. 128 and 93. The results indicate that lithium has no particular effect on the recovery of boron.

Inasmuch as some optical glasses contain lead, as well as barium and zinc, samples of such glasses were fused with sodium carbonate and a standardized boric acid solution was added. The range covered by these glasses was zinc oxide (0 to 8 percent), barium oxide (0 to 27 percent), and lead oxide (0 to 70 percent). The results by the recommended procedure are given in table 2.

TABLE 2.—*Determination of* B_2O_3 *in optical glasses containing barium, lead, and zinc*

Glass used contained—				B_2O_3 added		Added B_2O_3 found	Difference
BaO	PbO	ZnO	B_2O_3				
%	%	%	%	g	%	%	%
31.6	None	7.5	3.77	0.0344	6.88	6.94	+0.06
40.7	None	7.7	5.82	.0275	5.50	5.64	+.14
None	None	1.8	11.16	.01376	2.75	2.76	+.01
14.9	23.8	8.0	None	.0362	7.24	7.12	−.12
				.0724	14.48	14.47	−.01
None	45.6	None	None	.0362	7.24	7.28	+.04
None	51.4	None	None	.0724	14.48	14.38	−.10
None	69.4	None	None	.0362	7.24	7.26	+.02
				.0724	14.48	14.53	+.05

The interference caused by fluorine has not yet been overcome.

III. RECOMMENDED METHOD OF ANALYSIS

1. REQUIRED REAGENTS

1. Paranitrophenol. One gram dissolved in 75 ml of ethanol (95 percent) and made up to 100 ml with distilled water.

2. Phenolphthalein. One gram dissolved in 100 ml of ethanol (95 percent) and made up to 200 ml with distilled water.
3. Hydrochloric acid, 0.5 *N*.
4. Sodium hydroxide, carbon dioxide and boron free, 0.5 *N*, and stored in containers free from boron.
5. Barium hydroxide, 0.1 *N*.
6. Ethanol, absolute.
7. Ethyl ether, aldehyde and peroxide free (as indicated by test given below).
8. Mannitol.

2. ANALYTICAL PROCEDURE

Mix 0.5000 g of glass and 1.000 g of sodium carbonate intimately together in a mortar and transfer to a platinum crucible. Fuse at as low a temperature as possible and only as long as is necessary to effect complete decomposition. Cool, wash the lower surface of the lid, catching the washings in the crucible, and wash down the inside of the crucible with hot distilled water. Disintegrate the melt on the bath with the aid of a platinum wire and concentrate to about 5 ml. Cool and neutralize most of the alkali with H_2SO_4 (approximately 5.8 *N*), guarding against loss of boron both by spattering and by volatilization. Add two drops of *p*-nitrophenol, complete neutralization dropwise, add 1 ml of H_2SO_4 (approximately 11.6 *N*), and transfer to a calibrated 100-ml glass-stoppered cylinder. Dilute to 25 ml, add 25 ml of absolute ethanol and 50 ml of ether,[3] and shake intermittently for 20 minutes, noting the temperature. Allow the layers to separate, record their volumes, transfer 50 ml of the ether layer to a 250-ml Erlenmeyer flask, add two drops of *p*-nitrophenol, and titrate to the *p*-nitrophenol end point with 0.5 *N* NaOH. Read the burette, add 1 ml of phenolphthalein, and continue the titration to the phenolphthalein end point. Add a volume of 0.5 *N* NaOH equal to three times that used between the two end points and shake the flask vigorously. Wash down the inside of the flask with distilled water and make the water volume up to 40 to 50 ml. Boil off the ether and ethanol as quickly as possible,[4] first on a water or steam bath and finally over a free flame. Adjust the volume to 35 to 45 ml, cool, and then make just acid with 0.5 *N* HCl. Warm on the steam bath for about 1 minute, and boil under reduced pressure until cool to remove CO_2. Titrate to the *p*-nitrophenol end point with 0.1 *N* Ba $(OH)_2$, record the burette reading, add mannitol, and titrate the B_2O_3. One milliliter of 0.1 *N* $Ba(OH)_2$ is equivalent to 0.00348 g of B_2O_3. A blank determination should be made using a glass, preferably similar to the ones being analyzed, but containing no boron (such as Standard Sample 80 of the National Bureau of Standards).

[3] It became evident during the course of this work that the quality of the ether used in this method must be examined at frequent intervals and should be purified if the *p*-nitrophenol end point is not sharp. A satisfactory test method is to treat approximately 50 ml of ether, 5 ml of 0.5 *N* NaOH, and about 50 ml of distilled water exactly as a sample would be treated for the removal of ether and ethanol. Upon diluting to 35 to 45 ml and neutralizing with 0.5 *N* HCl to the *p*-nitrophenol end point, no color should be evident. For purification, a modification of the method of S. Palkin and H. R. Watkin (Ind. Eng. Chem. **21**, 863 (1929)) was used. The ether was first agitated with asbestos impregnated with alkaline permanganate (5 ml of saturated $KMnO_4$ to 15 ml of 33-percent NaOH) and then slowly siphoned, in a very fine stream, through a column of the alkaline permanganate and into a bottle. This bottle also contained asbestos impregnated with alkaline permanganate and was kept in a cool, dark place.

[4] Long standing on the water or steam bath gives a colored acid solution, which has a tendency to mask the *p*-nitrophenol end point in the final titration.

The equation for calculating the percentage of B_2O_3 in a 0.5000-g sample is as follows:

$$\text{Percentage of } B_2O_3 = 4(B_2O_3)_{50\,Et_2O}\left[V_{Et_2O} + \frac{V_{H_2O}}{k}\right],$$

where $(B_2O_3)_{50\ Et_2O}$ represents the grams of B_2O_3 in the 50-ml aliquot portion taken for analysis, and V_{Et_2O} and V_{H_2O} are the volumes of the ether layer and the water layer, respectively. The value of k in the above is found from the equation

$$k = 0.417 - 0.00232t.$$

By means of this method, it has been possible to determine amounts of B_2O_3 as low as 0.30 and as high as 16.00 percent.

IV. CONCLUSION

The substitution of sulfuric acid for the hydrochloric acid originally recommended overcomes the interference of certain elements, such as barium, iron, and zinc, and gives results no less satisfactory. Although it is not known whether or not lead interferes in the original method, it does not in the present modification. It is thought that the method described above should be substituted for the one originally described by us, when one has occasion to determine boron in a glass of unknown composition or when barium, iron, zinc, or lead is present.

WASHINGTON, May 13, 1941.

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SEPARATION OF HYDROCARBONS BY AZEOTROPIC DISTILLATION [1*]

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ABSTRACT

The following topics are discussed: the principles involved in azeotropic distillation; the substances that form azeotropic mixtures with hydrocarbons; the separation of hydrocarbons by azeotropic distillation, including aromatics from naphthenes and paraffins, naphthenes from paraffins, aromatic hydrocarbons having different numbers and kinds of rings, naphthene hydrocarbons having different numbers of naphthene rings, olefin hydrocarbons, and isomeric hydrocarbons; the differences in the change of boiling point with pressure for the different types of hydrocarbons; and the benefits of distillation at reduced pressure prior to azeotropic distillation at the normal operating pressure. A general procedure is outlined for separating a given (gasoline or kerosene) fraction of petroleum by means of distillation alone in its several variations (at the normal distilling pressure, at reduced pressure, and with an azeotrope-forming substance). A list is given of the hydrocarbons which are known to be separable in a substantially "pure" state by distillation alone from petroleum or its products.

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I. INTRODUCTION

In the work of the American Petroleum Institute Research Project 6 on the chemical constitution of petroleum, the separation of pure hydrocarbon compounds is accomplished by the appropriate inter-

[1] This investigation is part of the work of Research Project 6 of the American Petroleum Institute, from whose research fund financial assistance has been received.

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* A condensed version of part of the material given in this paper was presented before the Refining Division of the American Petroleum Institute at Chicago, Ill., on November 13, 1940, and published in the Proceedings of the American Petroleum Institute 21(III), 43 (1940) under the title "Use of Azeotropic Distillation in Separating Hydrocarbons from Petroleum."

locking of the fractionating processes of distillation, crystallization, extraction, and adsorption. Each of these processes has one or more variations: distillation may be performed at the fixed "normal" operating pressure,[3] or by alternate distillation at the normal and at reduced pressure, or by distillation with an azeotrope-forming substance; crystallization may be performed by simple refrigeration of the mixture of hydrocarbons followed by equilibrium melting with centrifuging, or by refrigeration of the mixture dissolved in an appropriate solvent followed by equilibrium melting with centrifuging; extraction may be performed with different solvents and at different temperatures; and adsorption may be performed with different adsorbents. Of the foregoing processes, distillation at a fixed pressure produces fractionation substantially according to size of molecules or molecular weight; whereas fractionation substantially according to type of molecule is produced by distillation at alternating different pressures, by distillation with an azeotrope-forming substance, by extraction, and by adsorption.

Although the most effective and complete results usually are obtained by utilizing all four processes of fractionation and their appropriate variations, it frequently may happen that distillation alone, in its several variations, may suffice to bring about a substantially complete separation of given hydrocarbons or of groups of isomeric hydrocarbons. When it is possible to use distillation alone, the laboratory or plant has the advantage of being able to utilize distillation apparatus or equipment that is already available and that has been developed to a high degree of efficiency for operations on both small and large scales.

In this paper are presented a discussion of azeotropic distillation and of substances that form azeotropic mixtures with hydrocarbons, a description of the separation of various kinds of hydrocarbons by azeotropic distillation, a discussion of the benefits of prior distillation at reduced pressure, an outline of a general procedure for separating a given gasoline or kerosene fraction of petroleum by using only distillation in its several variations, and a list of hydrocarbons known to be separable by distillation alone.

II. PRINCIPLES INVOLVED IN AZEOTROPIC DISTILLATION

In the ideal liquid solution, the partial vapor pressure, p_i, of a given component, i, is equal to the mole fraction, N_i, of component i multiplied by the vapor pressure, $p_i°$, of component i in the pure state at the given temperature:

$$p_i = N_i p_i°. \tag{1}$$

The total vapor pressure, P, is the sum of the several partial vapor pressures:

$$P = \Sigma p_i. \tag{2}$$

[3] As used in this paper, "normal" pressure refers not to atmospheric pressure but to that pressure at which the distillation of material of a given boiling range normally is performed, and reduced pressure refers to a pressure below that which is normal for the given material. In this laboratory, for example, normal pressure is 770 mm Hg for material boiling up to about 130° C at atmospheric pressure, 217 mm Hg for material boiling from about 130° to 200° C at atmospheric pressure, and 56.8 mm for material boiling from about 200° C to 260° C at atmospheric pressure. For hydrocarbons of the gasoline and kerosene ranges, the lowering of the boiling point between 770 and 217 mm and between 217 and 56.8 mm is 40° to 45° C.

For an ideal solution of two components, A and B, the partial vapor pressures are:

$$p_A = N_A p_A^\circ \quad (3)$$

$$p_B = N_B p_B^\circ = (1 - N_A) p_B^\circ \quad (4)$$

and the total vapor pressure is:

$$P = p_A + p_B = p_B^\circ + N_A(p_A^\circ - p_B^\circ). \quad (5)$$

The relations given by eq 3, 4, and 5, each of which is linear with the mole fraction, are shown graphically in part I of figure 1.

The laws of the ideal solution, as expressed by the foregoing equations, imply that, in the ideal solution of two components A and B, the interaction between the unlike molecules is the same as that between the like molecules.

In many real binary solutions, it is found that the interaction between the unlike molecules is not sigificantly different from the interaction between the like molecules, and the resulting partial and total vapor pressures are subtantially in accord with the relations holding for the ideal solution. Two such real binary solutions that are substantially ideal are the solution of ethylene bromide (1,2-dibromoethane) and propylene bromide (1,2-dibromopropane), the vapor pressures of which [1] [4] are illustrated in part II of figure 1, and the solution of *n*-heptane and methylcyclohexane, the vapor pressures of which [2, 3] are illustrated in part III of figure 1. In each of these cases, the respective components are very similar in molecular character.

Deviations from the laws of the ideal solution will occur when the interaction between unlike molecules is substantially different from that between like molecules. In the absence of other disturbing influences, such as the formation of hydrogen bonds, one property that may be used, within appropriate limits, to predict whether two given components may form a nearly ideal solution is that of the dipole moment of the molecules, which measures their polarity. Excluding components that form hydrogen bonds, substances of like polarity will be expected to form solutions in which the departures from ideality are not large. As summarized by Hildebrand [4]: "The total intermolecular field (in solutions of nonelectrolytes) includes contributions from the following effects: The potential between permanent electric dipoles; the potential between a permanent dipole and the temporary dipole it may induce in another molecule; the potential of actual chemical or electron pair bonds that may be formed; the potential due to the interaction always occurring between electron systems, regardless of the presence or absence of the foregoing effects . . . It is becoming evident, again, that the term 'association,' under which we have lumped all departures from normal behavior, must be subdivided into association arising from the interaction of dipoles and that due to the formation of definite chemical bonds. Of these [latter], perhaps the most interesting are the hydrogen bonds, or 'bridges' between oxygen, nitrogen, or fluorine atoms [which is] a species of chemical interaction. Substances containing hydroxyl [such as water and the alcohols], carboxyl [such as the ketones and aldehydes], or amino groups [such as aniline and other amines] show a type of association markedly

[4] Figures in brackets indicate the literature references at the end of this paper.

different from that of most other dipoles, such as those due to halogens." (For a detailed discussion of real solutions and their departures from ideality, see references [4, 5, 6, 7, 8, 9, 10].)

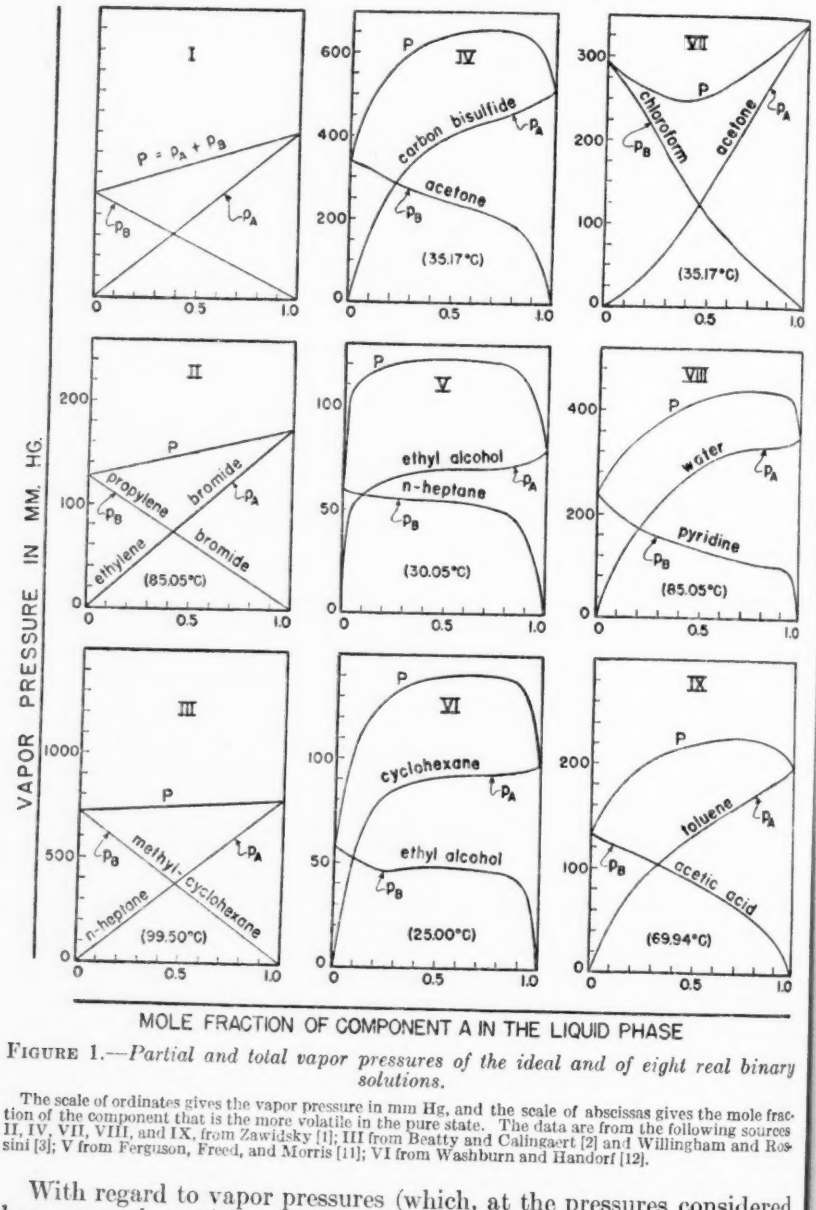

FIGURE 1.—*Partial and total vapor pressures of the ideal and of eight real binary solutions.*

The scale of ordinates gives the vapor pressure in mm Hg, and the scale of abscissas gives the mole fraction of the component that is the more volatile in the pure state. The data are from the following sources II, IV, VII, VIII, and IX, from Zawidsky [1]; III from Beatty and Calingaert [2] and Willingham and Rossini [3]; V from Ferguson, Freed, and Morris [11]; VI from Washburn and Handorf [12].

With regard to vapor pressures (which, at the pressures considered here, are substantially equal to the respective fugacities and, therefore, are a true measure of the escaping tendency of the components

from the given solutions), departures of real solutions from the ideal behavior are manifested as an increase or decrease in the partial vapor pressures from what would be their ideal values for the given concentrations, and as a corresponding increase or decrease in the total vapor pressure.

In figure 1, part IV gives the partial and the total vapor pressures for the binary solution of acetone and carbon disulfide [1] and part VII gives the same data for the binary solution of acetone and chloroform [1]. It is interesting to note that acetone forms with carbon disulfide a mixture having a total vapor pressure that is greater than that of carbon disulfide alone, which is the more volatile pure component, and hence there results a minimum-boiling azeotropic mixture. On the other hand, acetone forms with chloroform a mixture having a total vapor pressure that is less than that of the less volatile pure component, which in this solution is chloroform, and there results a maximum-boiling azeotropic mixture.

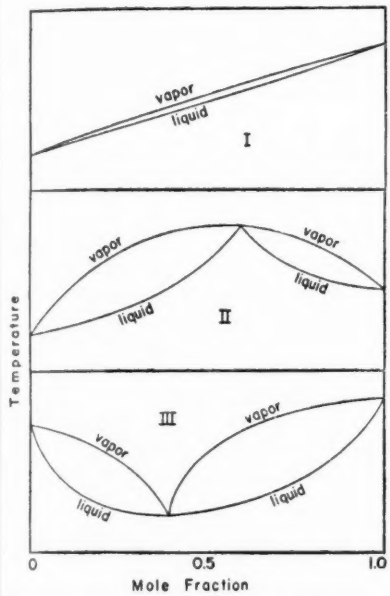

FIGURE 2.—*General temperature-composition diagrams for the liquid and vapor phases of the three types of binary solutions in which the components are completely soluble in one another.*

The scale of ordinates gives the temperature, and the scale of abscissas gives the mole fraction of that component which is the less volatile in the pure state. Part I represents ideal and other solutions in which the boiling point is, for all concentrations, always intermediate between the boiling points of the two pure components. Part II represents solutions in which there occurs a concentration for which the total vapor pressure is less than that of the less volatile pure component, forming a maximum-boiling azeotropic mixture. Part III represents solutions in which there occurs a concentration for which the total vapor pressure is greater than that of the more volatile pure component, forming a minimum-boiling azeotropic mixture.

Part VIII of figure 1 gives the vapor-pressure relations for the binary solution of water and pyridine [1], which system exhibits marked deviations from ideality and yields a mixture having a total vapor pressure greater than that of the more volatile pure component, and there results a minimum-boiling azeotropic mixture. In figure 1, parts V, VI, and IX show the partial and total vapor pressures for three binary systems, in which one component is respectively a paraffin, a naphthene, and an aromatic hydrocarbon: part V, *n*-heptane and ethyl alcohol [11]; part VI, cyclohexane and ethyl alcohol [12]; part IX, toluene and acetic acid [1]. In each of these systems, there occurs a mixture having a total vapor pressure greater than that of the more volatile pure component, which results in a minimum-boiling azeotropic mixture. For these systems, the departures from ideality are exceedingly large and yield in each case an azeotropic mixture having a boiling point considerably below that of the lower-boiling component.

For binary solutions in which the two components are completely miscible in the liquid phase, there are three possible kinds of temperature-composition diagrams (including both the liquid and vapor

phases), as illustrated in figure 2. Part I of figure 2 is illustrative of all ideal or other solutions in which the total vapor pressure is never less than that of the less volatile pure component nor greater than that of the more volatile pure component. That is to say, for this class of solutions, the boiling point of the liquid phase is for every concentration always intermediate between the boiling points of the two pure components. The binary systems illustrated in parts I, II, and III of figure 1 fall in this class. Part II of figure 2 is illustrative of those solutions in which there occurs a composition for which the total vapor pressure is less than that of the less volatile pure component, yielding a maximum-boiling mixture. The binary system illustrated in part VII of figure 1 falls in this class. Part III of figure 2 is illustrative of those solutions in which there occurs a composition for which the total vapor pressure is greater than that of the more volatile pure component, yielding a minimum-boiling azeotropic mixture. The binary systems illustrated in parts IV, V, VI, VIII, and IX of figure 1 fall in this class.

One characteristic feature of the temperature-composition diagrams for those systems forming azeotropic mixtures is that, except at the composition of the constant-boiling mixture, the spread in composition between the liquid and vapor phases is usually much greater than it is in systems that do not form azeotropic mixtures. This difference in the shapes of the liquid-vapor curves for the two kinds of solutions is illustrated in figure 3, which gives the temperature-composition diagrams for the binary system of benzene and *n*-hexane [13] and for the binary system of benzene and ethyl alcohol [14]. The much greater spread in composition (except for the constant-boiling mixture) between the liquid and vapor phases for the latter system than for the former is readily apparent. The separation of the azeotropic mixture of benzene and ethyl alcohol from either pure benzene or pure ethyl alcohol may be accomplished with distillation apparatus of relatively small separating efficiency.

III. AZEOTROPIC MIXTURES WITH HYDROCARBONS

With some exceptions, nearly all polar organic molecules of the proper volatility form, with hydrocarbons of the paraffinic, naphthenic, aromatic, and olefinic classes, mixtures that have a total vapor pressure greater than that of the more volatile pure component, yielding minimum-boiling azeotropic mixtures. The organic compounds that form azeotropic mixtures with these hydrocarbons include those containing hydroxyl, carboxyl, cyanide, amino, nitro, and other structural groups that tend to produce polarity in organic molecules.[5]

For a substantially complete list of the data that have been pub-

[5] All of the binary azeotropic mixtures in which one component is a hydrocarbon that have come to the attention of the authors are minimum-boiling with the exception of one system, that of pentachloroethane and 1,3,5-trimethylbenzene, which is a maximum-boiling one. It is likely that pentachloroethane will form maximum-boiling azeotropic mixtures with other hydrocarbons. Pentachloroethane differs from most of the other substances that form azeotropic mixtures with hydrocarbons (for which data are available) in that the polarity of the molecules in the latter class is produced by introducing an $-OH$, or $C=O$, or $-CN$, or similar group, into a molecule that is otherwise hydrocarbon; whereas the polarity of the pentachloroethane molecule is produced by introducing a $C-H$ bond into a molecule that otherwise is all composed of $C-Cl$ bonds. Hexachloroethane is substantially nonpolar.

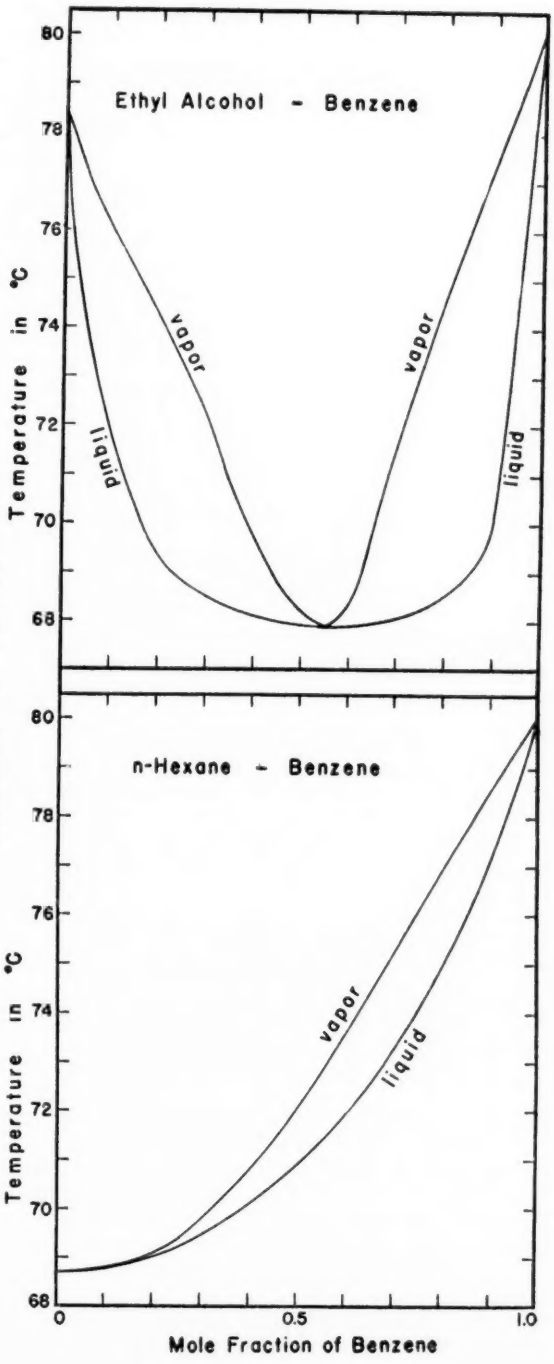

FIGURE 3. — *Temperature-composition diagrams for both the liquid and vapor phases for the binary solutions of ethyl alcohol and benzene and of n-hexane and benzene at a pressure of 1 atmosphere.*

The scale of ordinates gives the temperature in degrees centigrade, and the scale of abscissas gives the mole fraction of benzene. The diagram for the system of ethyl alcohol and benzene was constructed from the data of Fritzweiler and Dietrich [14] and that for the system of *n*-hexane and benzene was constructed from the data of Tongberg and Johnston [13].

lished on azeotropic mixtures having a hydrocarbon as one component, the reader is referred to the publications of Lecat [23, 24, 25, 26].

On the basis of the existing data on the azeotropic mixtures of hydrocarbons of the various types, together with the experimental data presented in section IV of this paper, the following conclusion may be drawn: For the five binary minimum-boiling azeotropic mixtures formed between a given azeotrope-forming substance and a paraffin, naphthene, monoolefin, diolefin, and aromatic hydrocarbon, respectively, with the five hydrocarbons having the same boiling point, the departure of the partial and total vapor pressures from ideality will be greatest for the paraffin azeotrope and least for the aromatic azeotrope, with the others intermediate and usually in the order given. In terms of the difference in boiling point between the lower-boiling pure component and the minimum-boiling azeotropic mixture, the lowering is greatest for the paraffin azeotrope and least for the aromatic azeotrope, with the others intermediate and usually in the order given. For a given azeotrope-forming substance and the three hydrocarbons, paraffin, naphthene, and aromatic, all boiling at the same temperature, the foregoing relations are illustrated schematically in figure 4.

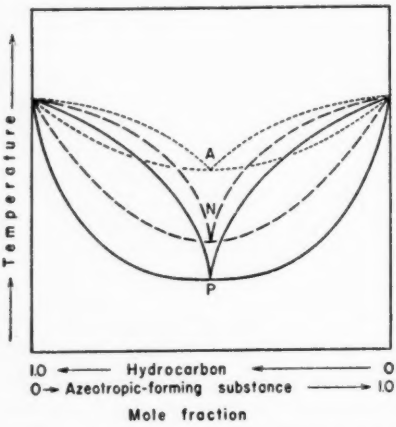

FIGURE 4.—*Schematic temperature-composition diagrams for both the liquid and vapor phases for binary solutions of a given azeotrope-forming substance with an aromatic hydrocarbon, a naphthene hydrocarbon, and a paraffin hydrocarbon, respectively, all having the same boiling point in the pure state at a given pressure.*

The scale of ordinates gives the temperature, and the scale of abscissae gives the mole fraction. These diagrams illustrate the differences to be expected in the boiling points of the binary azeotropic mixtures formed between a given azeotrope-forming substance and hydrocarbons of the aromatic, naphthene, and paraffin classes, all of the same normal boiling point. The compositions of such azeotropic mixtures will usually be somewhat but not greatly different. (In this diagram, the compositions of the azeotropic mixtures are given the same value for convenience in illustration.)

Figure 5 illustrates how, with a given azeotrope-forming substance, ethyl alcohol, the boiling point and the composition of the azeotropic mixture changes with the boiling point of the members of a given homologous series of hydrocarbons. Data are given for isopentane, *n*-pentane, *n*-hexane, *n*-heptane, and *n*-octane of the paraffins, for cyclohexane and methylcyclohexane of the naphthenes, for 3-methylbutene-1 and 2-methylbutene-2 of the monoolefins for 2-methylbutadiene-1,3 and hexadiene-1,5 for the diolefins, and for benzene and toluene of the aromatics [24]. When the boiling point of the hydrocarbon is considerably below that of the azeotrope-forming substance, the composition of the azeotrope mixture will be high in the given hydrocarbon, as shown for the two pentanes and the two pentenes. As the boiling point of the hydrocarbon increases, the boiling point of the azeotropic mixture, always below that of the hydrocarbon, moves toward the boiling point of the pure azeotrope-forming substance, and the composition of the azeotropic mixture likewise becomes leaner in hydrocarbon, with both the boiling point and the composition approaching those of the pure azeotrope-forming substance as a limit. Since this is the general behavior of

such systems, the dashed line, giving the loci of the points representing the boiling point and composition of the binary azeotropic mixtures of ethyl alcohol with the paraffin hydrocarbons listed, can be used to estimate the boiling point and composition of binary azeotropic

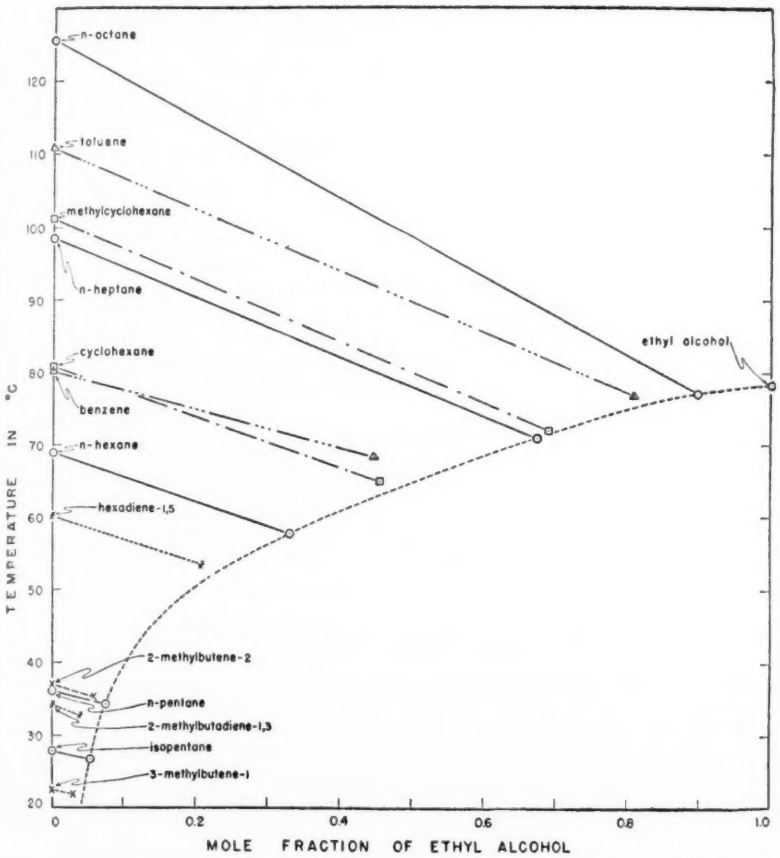

FIGURE 5.—*Plot of the values of the boiling point and composition of the binary azeotropic mixtures formed between ethyl alcohol and five paraffin hydrocarbons, two naphthene hydrocarbons, two monoolefin hydrocarbons, two diolefin hydrocarbons, and two aromatic hydrocarbons, respectively, together with the boiling points of the pure components.*

The scale of ordinates gives the temperature in degrees centigrade, and the scale of abscissas gives the mole fractions of ethyl alcohol in the respective azeotropic mixtures. The straight lines are drawn to join the boiling point and composition of the azeotropic mixture with the boiling point of the corresponding hydrocarbon in the pure state. The curved dashed line gives the loci of the points representing the boiling points and compositions of the binary azeotropic mixtures of ethyl alcohol with isopentane, *n*-hexane, *n*-heptane, and *n*-octane. The data are from Lecat [24]. (See text for discussion).

mixtures of ethyl alcohol with other paraffin hydrocarbons of known boiling point for which no data on azeotropic mixtures exist. It will be noted that the points for the other hydrocarbons fall above the line for the paraffin hydrocarbons approximately in the order naphthene, monoolefin, diolefin, and aromatic. It appears that, with a given

azeotrope-forming substance, all hydrocarbons of about the same boiling point will form azeotropic mixtures having roughly the same composition in terms of mole fractions of the azeotrope-forming substance, and that the boiling points of these same azeotropic mixtures usually will decrease in the order aromatic, diolefin, monoolefin, naphthene, and paraffin. The same general relations hold for the hydrocarbons with other azeotrope-forming substances, for which diagrams similar to figure 5 may also be prepared.

Figure 6 illustrates how with a given hydrocarbon, benzene, the boiling point and composition of the azeotropic mixture changes with

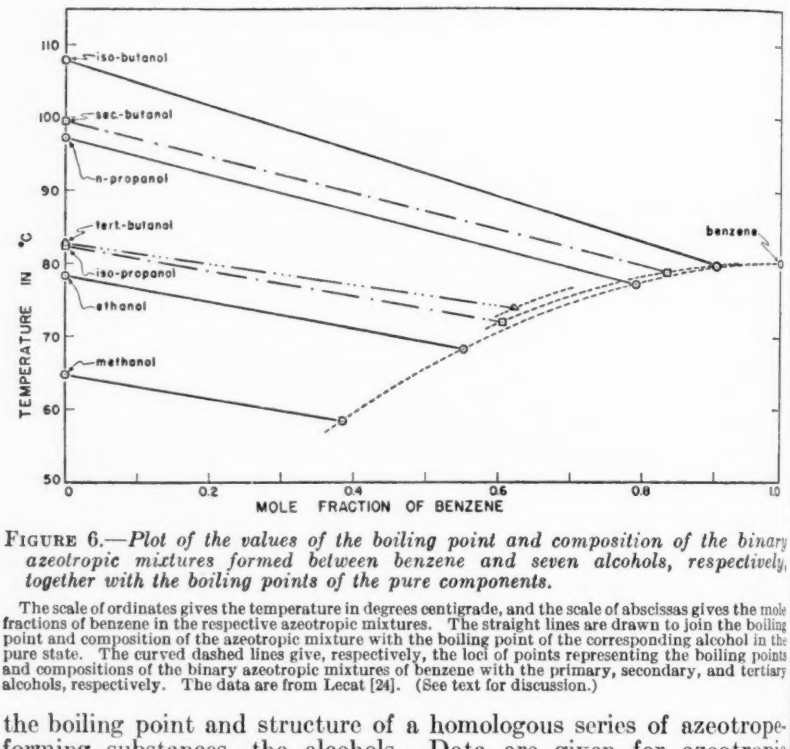

FIGURE 6.—*Plot of the values of the boiling point and composition of the binary azeotropic mixtures formed between benzene and seven alcohols, respectively, together with the boiling points of the pure components.*

The scale of ordinates gives the temperature in degrees centigrade, and the scale of abscissas gives the mole fractions of benzene in the respective azeotropic mixtures. The straight lines are drawn to join the boiling point and composition of the azeotropic mixture with the boiling point of the corresponding alcohol in the pure state. The curved dashed lines give, respectively, the loci of points representing the boiling points and compositions of the binary azeotropic mixtures of benzene with the primary, secondary, and tertiary alcohols, respectively. The data are from Lecat [24]. (See text for discussion.)

the boiling point and structure of a homologous series of azeotrope-forming substances, the alcohols. Data are given for azeotropic mixtures of benzene with methyl alcohol, ethyl alcohol, *n*-propanol, *iso*-propanol, *iso*-butanol, *sec*-butanol, and *tert*-butanol [24]. For benzene with the primary alcohols, increase in the boiling point of the alcohols produces an increase in the boiling point of the azeotropic mixture and a shift in composition toward higher content of hydrocarbon. In the limit of high-boiling alcohols, the boiling point and composition of the azeotropic mixture will approach those of pure benzene as a limit. It is also interesting to note the effect of the structure of the alcohol on the location of the line representing the boiling point and composition of the azeotropic mixtures. The lowest dashed line represents azeotropic mixtures of benzene with the primary alcohols, the next higher dashed line represents the secondary

alcohols, and the highest dashed line is drawn (on the basis of one point for the *tert*-butanol) to represent the tertiary alcohols. The departure from ideality is seen to be greatest for the primary alcohols, and least for the tertiary alcohols. Similar diagrams may be prepared for other hydrocarbons and other homologous series of azeotrope-forming substances.

IV. SEPARATION OF HYDROCARBONS BY AZEOTROPIC DISTILLATION

1. GENERAL DISCUSSION

In the work of the API Research Project 6 on the separation of hydrocarbons by azeotropic distillation in the laboratory, it is usually required of an azeotrope-forming substance that it

(a) have a boiling point near (not more than 30° or 40° C away from) the boiling range of the hydrocarbons to be separated;

(b) be completely soluble in water and preferentially less soluble in the hydrocarbon at room temperature, in order that the removal of the azeotrope-forming substance from the hydrocarbon with which it is associated in the azeotropic distillate may be accomplished easily by extraction with water;

(c) be completely soluble in the hydrocarbon at the distillation temperature, and for some degrees below this, in order that two phases shall not form in the condenser and reflux regulator and thus cause difficulty in the operation of the latter, and, where packed instead of bubble-cap columns are used, in order that the liquid phase in the rectifying section shall be entirely homogeneous, thus avoiding the possibility of irregular segregation of two liquid phases in the packed section;

(d) be readily obtainable in a sufficiently pure state at reasonable cost; and

(e) be nonreactive with hydrocarbons or with the material of the still.

Among the large number of substances that are available for use in the azeotropic distillation of hydrocarbons, those that have been used in this laboratory include the following compounds, which alone boil approximately at the temperatures given:

Compound	Boiling point at 1 atm
	° *C*
Methyl alcohol [15, 16]	66
Ethyl alcohol [15]	78
Methyl cyanide [6]	82
Acetic acid [17, 18, 19]	118
Ethylene glycol monomethyl ether [6]	125
Ethylene glycol monoethyl ether [6]	135
Ethylene glycol monomethyl ether acetate [6]	144
Ethylene glycol monobutyl ether [20] [6]	171
Diethylene glycol monomethyl ether [21] [6]	194

[6] See figures 7, 8, and 9 of this paper.

In preparing for the separation of the hydrocarbons occurring in any given fraction of petroleum, it is necessary to have the mixture of hydrocarbons well separated by a systematic and efficient distillation into a series of substantially constant-boiling fractions, prior to the azeotropic distillation. This is necessary in order to avoid having any paraffins mixed with lower-boiling naphthenes and, in turn, any paraffins and naphthenes mixed with lower-boiling aromatics, which kind of mixing will tend to nullify the separation normally attainable by the azeotropic distillation of mixtures of hydrocarbons of the same boiling point.

In connection with the selection of a suitable azeotrope-forming substance, there must be considered, in addition to the requirements already mentioned, the volume of hydrocarbon material that is to be distilled in one charge and the relation of this volume to the capacity and hold-up of the distillation column. If the available narrow-boiling charge is relatively small in volume, then it is best to select an azeotrope-forming substance boiling 30° to 40° C below the hydrocarbon material, in order that the concentration of hydrocarbon in the azeotropic distillate may be small.

For most of the work in this laboratory, it has been found desirable to use an azeotrope-forming substance whose boiling point is 0° to 30° C below that of the hydrocarbon material to be separated. The composition of the azeotropic distillate usually will range from about 0.2 to 0.6 mole fraction of hydrocarbon. As can be seen from figures 5 and 6, the use of a higher-boiling or lower-boiling azeotrope-forming substance will result in an azeotropic distillate having, respectively, a higher or lower concentration of hydrocarbon. Unless there is some restriction as to the volume of the still pot in reference to the volume of hydrocarbon to be charged, and unless there is some limitation as to the time available for the distillation of a given lot of material, it is usually best to use an amount of azeotrope-forming substance in excess of the amount required to carry all of the hydrocarbon material out of the distillation column as azeotropic distillate.

In the following parts 2 to 5 of this section are presented the results of azeotropic distillations each performed as a single batch distillation in a column having a separating efficiency equivalent to from 90 to 100 theoretical plates. Ten of the eleven sets of data are new.

2. SEPARATION OF AROMATICS FROM NAPHTHENES AND PARAFFINS

Figure 7 illustrates the results obtained in separating aromatic hydrocarbons from naphthenes and paraffins by azeotropic distillation, the separation being indicated by the change in refractive index of the hydrocarbon portion of each fraction of distillate with the amount of hydrocarbon recovered.

Part I of figure 7 shows the separation obtained in the azeotropic distillation, with methyl cyanide (boiling point 82° C), of a mixture of toluene (boiling point 110.6° C) with a substantially constant-boiling (110.0° to 110.5° C) fraction of petroleum containing paraffin hydrocarbons with some naphthenes. The separation of toluene from these paraffin and naphthene hydrocarbons having substantially the same boiling point is almost quantitative.

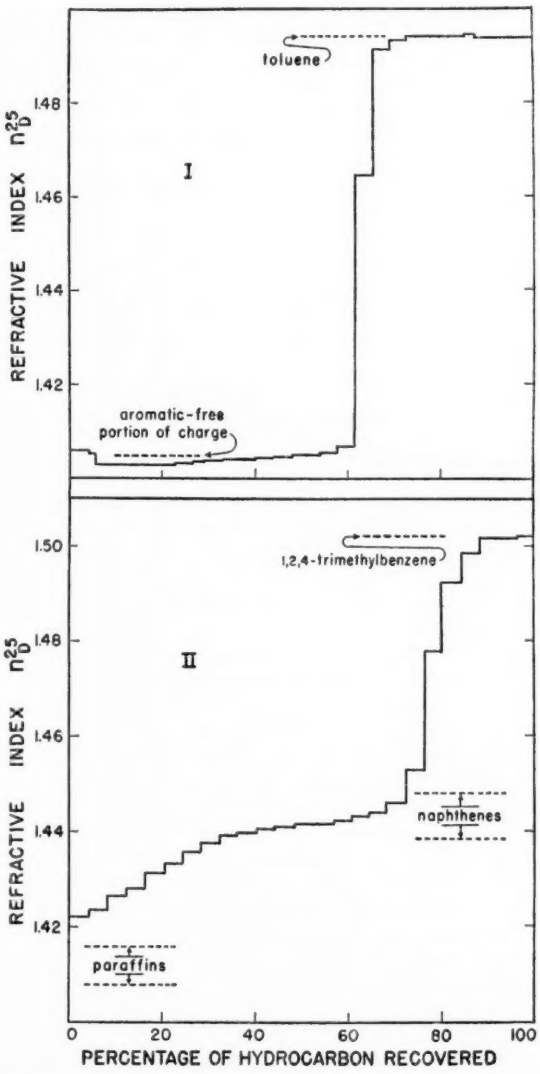

FIGURE 7.—*Diagrams illustrating the separation of aromatic hydrocarbons from naphthenes and paraffins by azeotropic distillation.*

The scale of ordinates gives the refractive index of the hydrocarbon recovered from the azeotropic distillate, and the scale of abscissas gives the percentage of the hydrocarbon recovered. The distillations were performed in a Bruun [36] glass bubble-cap column, of 138 rectifying units, equipped with a special pot, head, and jacket assembly [3], with a reflux ratio of 40 or 50 to 1, a rate of removal of distillate of 20 to 25 ml per hour, and a total time of distillation of about 100 hours.

Part I shows the results of the azeotropic distillation, with methyl cyanide at atmospheric pressure, of a mixture of toluene (boiling point 110.6° C) with a narrow-boiling (110.0° to 110.5° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 20 percent by volume of aromatics and about 35 percent of paraffins and naphthenes, with the latter two forming a two-phase mixture with methyl cyanide at the distillation temperature.

Part II shows the results of the azeotropic distillation, with ethylene glycol monobutyl ether at 770 mm Hg, of the mixture of aromatic, naphthene, and paraffin hydrocarbons naturally occurring in a narrow-boiling (166° to 169° C) fraction of Midcontinent petroleum. The azeotropic distillate contained about 60 percent by volume of hydrocarbon, increasing from aromatic to naphthene to paraffin.

Part II of figure 7 shows the separation obtained in the azeotropic distillation, with ethylene glycol monobutyl ether (boiling point 171° C), of a narrow-boiling (166° to 169° C) fraction of petroleum containing aromatic, naphthene, and paraffin hydrocarbons [20]. There is noted here the almost complete separation of the aromatic hydrocarbons from the naphthenes and paraffins, and also a partial separation of the paraffins from the naphthenes.

Bruun and Hicks-Bruun [15] have described the use of methyl and ethyl alcohols in the separation of that fraction of petroleum normally boiling between 54° and 75° C, which contained the four higher-boiling hexanes, cyclohexane, methylcyclopentane, and benzene.

Rose and White [19] have described in detail the separation of the aromatic hydrocarbons from narrow-boiling fractions of petroleum of the range 154° to 162° C by azeotropic distillation with acetic acid (boiling point 118° C).

Tongberg and Johnston [13] have described the separation of benzene from *n*-hexane by azeotropic distillation with *tert*-butanol.

3. SEPARATION OF NAPHTHENES FROM PARAFFINS

Figure 8 illustrates the results obtained in separating naphthene hydrocarbons from paraffin hydrocarbons by azeotropic distillation.

Part I of figure 8 shows the separation obtained in the azeotropic distillation, with methyl alcohol (boiling point 66° C), of a substantially constant-boiling (92° C) fraction of paraffin and naphthene hydrocarbons [16].

Part II of figure 8 shows the separation obtained in the azeotropic distillation, with ethylene glycol monomethyl ether acetate (boiling point 144° C), of a narrow-boiling (157° to 158° C) fraction of petroleum containing paraffins and naphthenes.

Part III of figure 8 shows the separation obtained in the azeotropic distillation, also with ethylene glycol monomethyl ether acetate (boiling point 143° C) of a narrow-boiling (177° to 178° C) fraction of petroleum containing paraffins and naphthenes.

Part IV of figure 8 shows the separation obtained in the azeotropic distillation, with ethylene glycol monobutyl ether (boiling point 171° C), of a narrow-boiling (167° to 168° C) fraction of petroleum containing paraffins and naphthenes [20].

Part V of figure 8 shows the separation obtained in the azeotropic distillation, with diethylene glycol monomethyl ether (boiling point 193° C), of a narrow-boiling (200° to 201° C) fraction of petroleum containing paraffins and naphthenes [21].

Schicktanz [17] has described in detail the separation of naphthenes from paraffins in a narrow-boiling (165° to 166° C) fraction of petroleum by azeotropic distillation with acetic acid (boiling point 118° C).

Figure 9 illustrates the results obtained in the distillation of the same narrow-boiling (157° to 158° C) mixture of paraffins and naphthenes with three different azeotrope-forming substances. The results shown by the dotted line were obtained with ethylene glycol monomethyl ether acetate (boiling point 144° C), those shown by the dashed line were obtained with ethylene glycol monoethyl ether (boiling point 135° C), and those shown by the solid line were obtained with ethylene glycol monomethyl ether (boiling point 125° C). The experiment with ethylene glycol monomethyl ether gave the best separation.

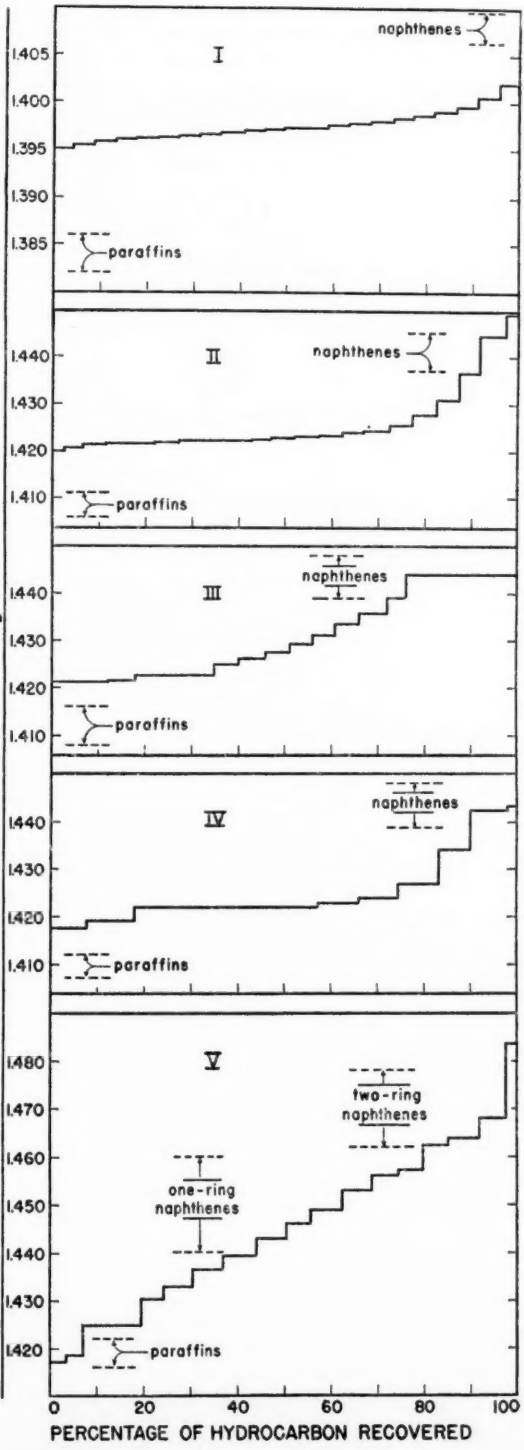

FIGURE 8.—*Diagrams illustrating the separation of naphthene hydrocarbons from paraffins by azeotropic distillation.*

The scale of ordinates gives the refractive index of the hydrocarbon recovered from the azeotropic distillate and the scale of abscissas gives the percentage of the hydrocarbon recovered. The distillations were performed in the following columns: Part I in a helix-packed column, with rectifying section 5/6×100 in., equivalent to about 90 theoretical plates; parts II and V, in helix-packed columns with rectifying sections 1×110 in. equivalent to about 100 theoretical plates [3]; parts III and IV in the Bruun bubble-cap column described under figure 7. The rate of removal of distillate was from 12 to 20 ml/hour, with a reflux ratio of 40 or 50 to 1 and a total time of distillation of about 100 hours.

Part I shows the results of the azeotropic distillation with methyl alcohol at atmospheric pressure, of a narrow-boiling (91.4° to 91.8° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 60 percent by volume of hydrocarbon.

Part II shows the results of the azeotropic distillation, with ethylene glycol monomethyl ether acetate at 770 mm Hg, of a narrow-boiling (157° to 158° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 40 percent by volume of hydrocarbon.

Part III shows the results of the azeotropic distillation, with ethylene glycol monomethyl ether acetate at 770 mm Hg, of a narrow-boiling (177° to 178° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 20 percent by volume of hydrocarbon.

Part IV shows the results of the azeotropic distillation, with ethylene glycol monobutyl ether at 770 mm Hg, of a narrow-boiling (167° to 168° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 70 percent by volume of hydrocarbon.

Part V shows the results of the azeotropic distillation, with diethylene glycol monomethyl ether at 770 mm Hg, of a narrow-boiling (200° to 201° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 55 percent by volume of hydrocarbon.

As contrasted with the separation of aromatic hydrocarbons from naphthenes and paraffins, which is a relatively easy fractionation, as shown in figure 7, the separation of naphthenes from paraffins is accomplished with greater difficulty, as indicated by the curves in figures 8 and 9. Whereas the complete separation of nearly all of the aromatic material may be accomplished by one azeotropic distillation in an efficient rectifying column, several such distillations, involving appropriate blending of the partially separated hydro-

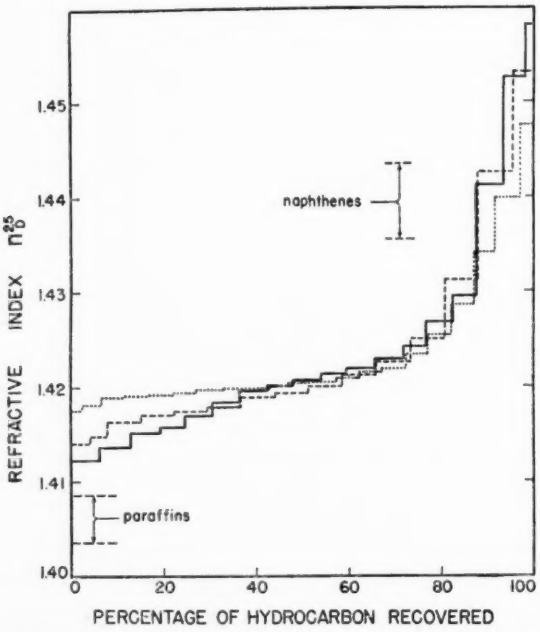

FIGURE 9.—*Diagram illustrating the separation of a given mixture of paraffins and naphthenes with three different azeotrope-forming substances.*

The scale of ordinates gives the refractive index of the hydrocarbon recovered from the azeotropic distillate, and the scale of abscissas gives the percentage of the hydrocarbon recovered. The distillations were performed in the Bruun bubble-cap column described under figure 7, with a rate of removal of distillate of about 15 ml/hour, a reflux ratio of about 50 to 1, and a total time of distillation of about 100 hours.

The dotted line, the dashed line, and the solid line show the results obtained with ethylene glycol monomethyl ether acetate, ethylene glycol monoethyl ether, and ethylene glycol monomethyl ether, respectively, on the same narrow-boiling (157° to 158° C) mixture of paraffins and naphthenes. The azeotropic distillate contained about 40 percent by volume of hydrocarbon.

carbons, are required to bring about the separation of the naphthenic material from the paraffinic.

4. SEPARATION OF AROMATIC HYDROCARBONS

Figure 10 illustrates the results obtained in separating various kinds of aromatic hydrocarbons from one another.

Part I of figure 10 shows the separation obtained in the azeotropic distillation, with diethylene glycol monomethyl ether at 217 mm Hg, of a narrow-boiling (204° to 206° C at 760 mm Hg) fraction of aromatic hydrocarbons from the kerosene portion of petroleum. There is indicated here the separation of mono,- di-, or tri-alkyl

benzene from 1,2,3,4-tetramethylbenzene, and in turn this latter from tetrahydronaphthalene. The latter separation results from the fact that one molecule has a benzene ring and the other is the partially

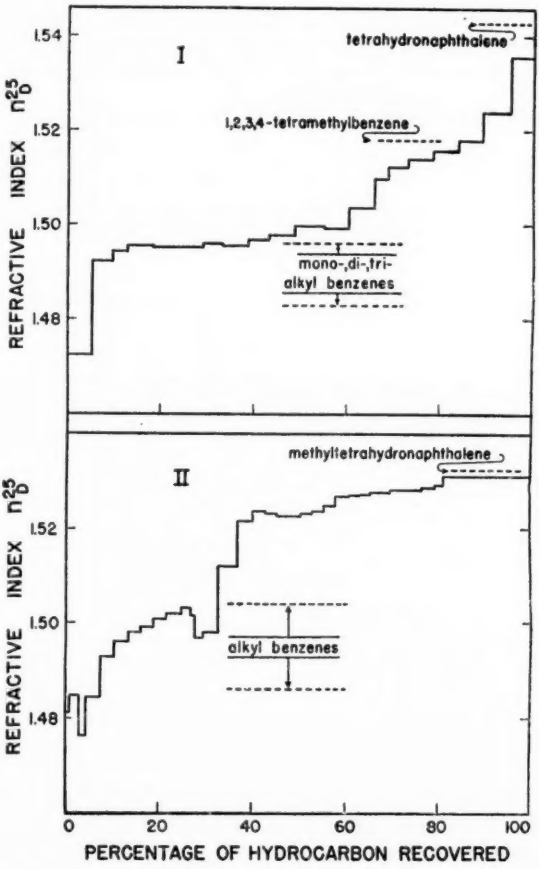

FIGURE 10.—*Diagrams illustrating the separation of different kinds of aromatic hydrocarbons from one another by azeotropic distillation.*

The scale of ordinates gives the refractive index of the hydrocarbon recovered from the azeotropic distillate, and the scale of abscissas gives the percentage of the hydrocarbon recovered. The distillations were performed in columns [3] similar to those for parts II and V under figure 8, at 217 mm Hg, with distillate removed at the rate of 10 to 20 ml/hour, with a reflux ratio of 40 or 50 to 1, and a total time of distillation of about 200 hours.

Part I shows the results of the azeotropic distillation, with diethylene glycol monomethyl ether at 217 mm Hg, of a narrow-boiling (204° to 206° C at 1 atm) mixture of aromatic hydrocarbons separated from the kerosene fraction of petroleum. The azeotropic distillate contained about 55 percent by volume of hydrocarbon.

Part II shows the results of the azeotropic distillation, with diethylene glycol monomethyl ether at 217 mm Hg, of a narrow-boiling (224° to 225° C at 1 atm) mixture of aromatic hydrocarbons separated from the kerosene fraction of petroleum. The azeotropic distillate contained about 25 percent by volume of hydrocarbon.

hydrogenated naphthalene composed of one aromatic ring doubly linked to a naphthene ring. The former separation is interesting in that it occurs between a molecule having a benzene ring with four alkyl radicals and a molecule having a benzene ring with one, two, or

three alkyl radicals, the molecules having about the same boiling point.

Part II of figure 10 shows the separation obtained in the azeotropic distillation, also with diethylene glycol monomethyl ether at 217 mm Hg, of a narrow-boiling (224° to 225° C) fraction of aromatic hydrocarbons from the kerosene portion of petroleum. There is indicated here the separation of alkyl benzenes from methyl tetrahydronaphthalene.

5. SEPARATION OF NAPHTHENE HYDROCARBONS

The separation of one-ring naphthenes fron two-ring naphthenes is indicated in part V of figure 8. Although no actual data are available, it appears that it should be possible to separate a monoalkyl cycloparaffin from a polyalkyl cycloparaffin normally boiling at the same temperature.

6. SUMMARY OF SEPARATIONS OBTAINABLE BY AZEOTROPIC DISTILLATION

From the data that are now available, the following conclusions may be drawn with regard to the separations obtainable by azeotropic distillation in distillation columns having separating efficiencies equivalent to from 50 to 100 theoretical plates:

(a) Aromatic hydrocarbons may be separated from naphthenes and paraffins with relative ease by one appropriate azeotropic distillation in an efficient rectifying column, in mixtures in which the aromatic hydrocarbons normally have boiling points not significantly lower than the boiling points of the naphthenes and paraffins in the given mixtures.

(b) Naphthene hydrocarbons may be separated from paraffins by several distillations with an appropriate azeotrope-forming substance in an efficient rectifying column, in mixtures in which the naphthene hydrocarbons normally have boiling points not significantly lower than the boiling points of the paraffins in the given mixtures.

(c) Aromatic hydrocarbons of different degrees of "aromaticity", resulting from different numbers and kinds of rings, as for example an alkyl benzene, an alkyl tetrahydronaphthalene, and an alkyl naphthalene, may be separated from one another, in mixtures in which the various aromatic hydrocarbons have substantially the same normal boiling points, or in which any differences in boiling point are of the same magnitude and sign as the differences in the boiling points of the corresponding binary azeotropic mixtures. Such separations may require one or more azeotropic distillations in an efficient rectifying column, depending upon the nature of the components of the mixture and its complexity.

(d) Naphthene hydrocarbons having different numbers of naphthene rings in the molecule may be separated from one another, in mixtures in which the various naphthene hydrocarbons have substantially the same normal boiling points, or in which the naphthenes with two rings have boiling points not significantly lower than the boiling points of the naphthenes with one ring. Several azeotropic distillations in an efficient rectifying column usually will be required for the substantially complete separation of such compounds.

The work of this laboratory has not included olefinic hydrocarbons because of their absence from natural petroleum. Nevertheless, on

the basis of the known data on their azeotropic mixtures, by analogy with the paraffins, naphthenes, and aromatics, and considering the work of Matuszak and Frey [34], who devised a procedure for separating the butanes and butenes by azeotropic distillation with sulfur dioxide, the following general conclusions may be drawn with regard to the separations obtainable in azeotropic distillations involving olefinic hydrocarbons in rectifying columns of from 50 to 100 theoretical plates:

(a) Monoolefin hydrocarbons may be separated with relative ease from aromatic hydrocarbons, from paraffin hydrocarbons, and perhaps as easily from diolefin hydrocarbons, but with less ease from naphthene hydrocarbons.

(b) Diolefin hydrocarbons may be separated with relative ease from naphthenes and paraffins, but with less ease from aromatics and monoolefins.

It may be possible to extend the method of azeotropic distillation to the separation of isomers of the same class and subclass of hydrocarbons, by appropriately selecting the azeotrope-forming substance and by using a highly efficient rectifying column. The curve shown in part I of figure 10 indicates that of two alkyl benzenes having about the same boiling point the one with the smaller number of alkyl groups will tend, with a given azeotrope-forming substance, to form a lower-boiling azeotropic mixture than the alkyl benzene with more alkyl groups. Furthermore, it should be possible to improve significantly the separation obtainable in the ordinary distillation in a given rectifying column of two isomers having the same number and kind of groups substituent to the main chain or ring of the molecule and boiling close together, by distilling them with an appropriate azeotrope-forming substance having a boiling point so low that the azeotropic distillate is low in hydrocarbon. Notwithstanding the fact that the difference in the boiling points of the respective azeotropic mixtures may be much less than the difference in the boiling points of the two isomers alone, this procedure may be successful because of the greater spread of the liquid-vapor lines of the temperature-composition diagram for the two azeotropic mixtures (considered as a binary system) than for the binary system of the two isomers alone.

In cases requiring the purification of a small quantity of a compound in a rectifying column having a capacity and hold-up much too large for the straight distillation of the given quantity of material, azeotropic distillation may be utilized for the purification. In this case, an azeotrope-forming substance can be selected which has a boiling point sufficiently below that of the given material that the azeotropic distillate may have any required low value of concentration of the given compound, and all of the latter then may be put through the still as distillate by using an excess of the azeotrope-forming substance. This method of purification will fail when the impurity is such that there is no significant difference in boiling point between its azeotropic mixture and that of the compound proper. However, in case a ternary azeotrope is formed between the compound, the impurity, and the azeotrope-forming substance, the impurity may be removed at the expense of losing a corresponding amount of the compound proper [28]. [7]

[7] For a discussion of the use of azeotropic distillation in producing water-free ethyl alcohol and "pure" dioxane on a commercial scale, see [29, 30, 31, 32, 33].

V. DISTILLATION AT REDUCED PRESSURE

1. CHANGE OF BOILING POINT WITH PRESSURE FOR DIFFERENT TYPES OF HYDROCARBONS

It is usually possible to separate a mixture of hydrocarbons of different types but of the same "normal" boiling point by distillation at a pressure reduced below, or elevated above, the "normal" operating pressure [22, 37], because of the fact that in general the different types of hydrocarbons show significantly different changes of the boiling point with pressure.

Figure 11 illustrates the changes of boiling point with pressure shown by several different types of hydrocarbons, referred to the

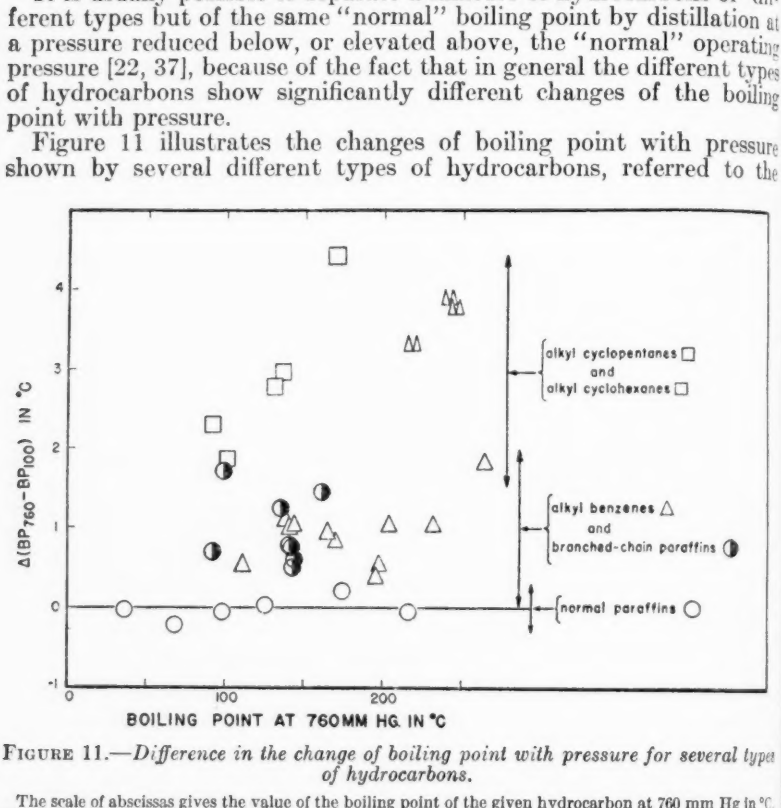

FIGURE 11.—*Difference in the change of boiling point with pressure for several types of hydrocarbons.*

The scale of abscissas gives the value of the boiling point of the given hydrocarbon at 760 mm Hg in °C. The scale of ordinates gives in °C the value of $\Delta\ (BP_{760}-BP_{100})=(BP_{760}-BP_{100})-(44.8+t/8-t^2/25{,}000)$. Here $BP_{760}-BP_{100}$ is the decrease in the boiling point of a given hydrocarbon as the pressure is reduced from 760 to 100 mm Hg; t is BP_{760}, the boiling point of the given hydrocarbon at 760 mm Hg; $44.8+t/8-t^2/25{,}000$ represents the value of $BP_{760}-BP_{100}$ for a hypothetical normal paraffin hydrocarbon having the same boiling point as the given hydrocarbon at 760 mm Hg; $\Delta\ (BP_{760}-BP_{100})$ is, therefore, the excess lowering of the boiling point of the given hydrocarbon over that of a hypothetical normal paraffin of the same boiling point at 760 mm Hg, as the pressure is reduced from 760 to 100 mm Hg. The various types of hydrocarbons are represented by the following symbols: ○, normal paraffins; ◑, branched-chain paraffins; □, naphthenes; △, alkyl benzenes; △△, naphthalene and methylnaphthalenes. The data were obtained from [3, 16, 20, 22, 28, 35, 38, 39, 40, 41]. See section V for discussion.

values for the normal paraffins as zero. For the normal paraffins with 5, 6, 7, 8, 10, and 12 carbon atoms per molecule, for 8 branched-chain paraffins, for 5 naphthene hydrocarbons, for 11 alkyl benzenes, and for naphthalene and the two methylnaphthalenes, there are plotted in figure 11 values of the quantity

$$\Delta\ (BP_{760}-BP_{100})=(BP_{760}-BP_{100})-(44.8+t/8-t^2/25{,}000).$$

Here t is BP_{760}, the boiling point of the given hydrocarbon at 760 mm Hg, $BP_{760}-BP_{100}$ is the lowering in boiling point of the given hydro-

carbon corresponding to a change in pressure from 760 to 100 mm Hg, and the quantity $44.8+t/8-t^2/25{,}000$ represents the value of the same property for a hypothetical normal paraffin having the same value of BP_{760}. The equation giving values of $BP_{760}-BP_{100}$ for the normal paraffins was deduced from the data on *n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, *n*-decane, and *n*-dodecane, and represents the existing data within 0.2° C, as can be seen from the values plotted for these normal paraffins. The quantity $\Delta(BP_{760}-BP_{100})$ represents, for a change in pressure from 760 to 100 mm Hg, the excess lowering of the boiling point of the given hydrocarbon over that of a hypothetical normal paraffin hydrocarbon having the same boiling point at 760 mm Hg.

On the basis of the data presented in figure 11, the following conclusions are drawn with regard to the value of $BP_{760}-BP_{100}$ for the branched-chain paraffins, the alkyl benzenes, the alkyl cyclopentanes and alkyl cyclohexanes, and naphthalene and its two methyl derivatives, referred to the values for the normal paraffins of the same boiling point at 760 mm Hg:

(a) For the branched-chain paraffins, the values of $BP_{760}-BP_{100}$ are greater than those for the corresponding normal paraffins by amounts ranging from near zero to about 2° C, with the values for the less highly branched paraffins apparently being smaller than those for the more highly branched paraffins.

(b) For the alkyl benzenes, the values of $BP_{760}-BP_{100}$ are greater than those for the corresponding normal paraffins by amounts ranging from near zero to about 2° C, which range is substantially the same as that for the branched-chain paraffins.

(c) For the alkyl cyclopentanes and alkyl cyclohexanes, the values of $BP_{760}-BP_{100}$ are greater than those for the corresponding normal paraffins by amounts which appear to range from near 1½° to about 4½° C.

(d) For naphthalene and its two methyl derivatives, the values of $BP_{760}-BP_{100}$ are greater than those for the corresponding normal paraffins by amounts lying within the range 3° to 4° C.

On the basis of the foregoing relations, it is evident that a distillation at reduced pressure prior to the azeotropic distillation at the "normal" pressure will enhance the separation obtainable in a mixture of paraffin hydrocarbons with alkyl cyclopentanes or alkyl cyclohexanes and in a mixture of alkyl benzenes with alkyl naphthalenes, but not in a mixture of alkyl benzenes with alkyl cyclopentanes or alkyl cyclohexanes.

2. BENEFITS OF DISTILLATION AT REDUCED PRESSURE PRIOR TO AZEOTROPIC DISTILLATION

It has been previously indicated (in section IV) that it is necessary to have the mixture of hydrocarbons well separated by an efficient regular distillation prior to the azeotropic distillation, in order to avoid having a mixing of the hydrocarbons of various types in a direction that will tend to nullify the separation obtainable by the azeotropic distillation. By the same line of reasoning, any preliminary fractionation that will produce a displacement of the hydrocarbons in the same direction as that to be produced by the azeotropic distillation will enhance the separation obtainable in the latter process.

Since distillation at reduced pressure serves to displace normally higher-boiling naphthenes into fractions containing normally lower-boiling paraffins, it follows that, where possible and practical, any mixture of naphthene and paraffin hydrocarbons should be distilled efficiently at a reduced pressure prior to the azeotropic distillation of the mixture at the "normal" distilling pressure. A similar procedure will enhance the separation of alkyl benzenes from naphthalene and alkyl naphthalenes.

The displacement with decrease in pressure of the boiling points of the aromatic hydrocarbons with respect to those of naphthene hydrocarbons is actually such that, if this mixture is distilled at reduced pressure prior to the azeotropic distillation at "normal" pressure, there results a small decrease in the separation obtainable by the azeotropic distillation; but this reversal is unimportant, because the separation of aromatics from naphthenes by azeotropic distillation is already extremely good. Furthermore, in the distillation of a mixture of hydrocarbons of the same boiling point but of different types, the aromatic hydrocarbons tend to be displaced with reference to the paraffins and naphthenes into lower-boiling fractions, because for the aromatics the ratio of vapor pressure to mole fraction is greater than it is for the naphthenes and paraffins in the same mixture.

In the separation of naphthenes from paraffins, which is in general the most difficult separation encountered in the fractionation of petroleum, the benefits obtainable from the distillation at reduced pressure prior to the azeotropic distillation are important and should be taken advantage of whenever possible.

VI. GENERAL PROCEDURE FOR SEPARATING HYDROCARBONS BY DISTILLATION ALONE

The general procedure for separating hydrocarbons from petroleum, by utilizing all of the fractionating processes of distillation, crystallization, extraction, and adsorption, has already been described [27]. It may frequently happen that apparatus for performing extraction and crystallization is not available, or that it is desired to simplify the fractionating procedure, as in connection with the economic requirements of large-scale operation. For such situations, the schematic diagram given in figure 12 illustrates the general procedure that may be followed in separating, by means only of distillation in its several variations, any given fraction of petroleum of the gasoline or kerosene ranges. Figure 12 and its legend are self-explanatory. It will be noticed that in the procedure outlined full advantage is taken of the benefits obtainable by distillation of the mixture of hydrocarbons at reduced pressure prior to their distillation azeotropically at the "normal" pressure.

While the foregoing procedure specifies only the hydrocarbons of the types occurring in petroleum, namely paraffins, naphthenes, and aromatics, appropriate modification of the procedure may be made easily to include the monoolefins and diolefins. For this purpose, there should be kept in mind the general order in which the different types of hydrocarbons arrange themselves with regard to their susceptibility to the process of azeotropic distillation, namely aromatic, diolefin, olefin, naphthene, and paraffin.

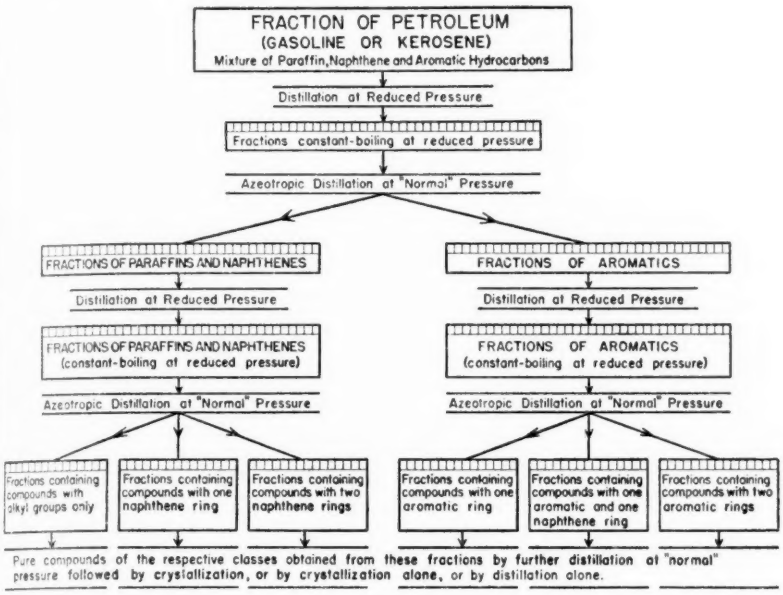
FRACTION OF PETROLEUM
(GASOLINE OR KEROSENE)
Mixture of Paraffin, Naphthene and Aromatic Hydrocarbons
Distillation at Reduced Pressure
Fractions constant-boiling at reduced pressure
Azeotropic Distillation at "Normal" Pressure
FRACTIONS OF PARAFFINS AND NAPHTHENES
FRACTIONS OF AROMATICS
Distillation at Reduced Pressure
Distillation at Reduced Pressure
FRACTIONS OF PARAFFINS AND NAPHTHENES (constant-boiling at reduced pressure)
FRACTIONS OF AROMATICS (constant-boiling at reduced pressure)
Azeotropic Distillation at "Normal" Pressure
Azeotropic Distillation at "Normal" Pressure
Fractions containing compounds with alkyl groups only
Fractions containing compounds with one naphthene ring
Fractions containing compounds with two naphthene rings
Fractions containing compounds with one aromatic ring
Fractions containing compounds with one aromatic and one naphthene ring
Fractions containing compounds with two aromatic rings
Pure compounds of the respective classes obtained from these fractions by further distillation at "normal" pressure followed by crystallization, or by crystallization alone, or by distillation alone.

FIGURE 12.—*Schematic diagram illustrating the separation of a fraction (gasoline or kerosene) of petroleum by distillation alone.*

The several variations of distillation employed in this procedure are at "normal" pressure, at reduced pressure, and with an azeotrope-forming substance. (See footnote 3, p. 40, for the meaning of "normal" pressure.)

VII. LIST OF HYDROCARBONS SEPARABLE BY DISTILLATION ALONE

By distillation alone (in its several variations of distillation at "normal" pressure, distillation at reduced pressure, and distillation with an azeotrope-forming substance), it is possible to separate the compounds, or mixtures, listed in table 1 from appropriate crude petroleums or from appropriate products of the refining process. A number of the substances, listed in table 1, are already being produced on a commercial scale from petroleum or petroleum products. These include methane, ethane, propane, isobutane, *n*-butane, isopentane, *n*-pentane, 2,2-dimethylbutane, mixed hexanes (54° to 71° C) mixed heptanes (80° to 99° C), mixed octanes (100° to 127° C), ethylene, propylene, mixed butenes, and concentrates of benzene, toluene, and "xylene."

TABLE 1.—*List of hydrocarbons separable from petroleum or petroleum products by distillation alone*

Formula	Name and type	Boiling point at 1 atm
	PARAFFINIC	
		°C
CH_4	Methane	−161.7
C_2H_6	Ethane	−88.6
C_3H_8	Propane	−42.2
C_4H_8	2-Methylpropane (isobutane)	−12.1
C_4H_8	*n*-Butane	−0.5
C_5H_{12}	2-Methylbutane (isopentane)	27.9
C_5H_{12}	*n*-Pentane	36.1
C_6H_{14}	2,2-Dimethylbutane (neohexane)	49.8
C_6H_{14}	2,3-Dimethylbutane	58.0
C_6H_{14}	2-Methylpentane	60.3
C_6H_{14}	3-Methylpentane	63.3
C_6H_{14}	*n*-Hexane	68.7
C_7H_{16}	Mixture of methylhexanes	90 to 92
C_7H_{16}	*n*-Heptane	98.4
C_8H_{18}	Mixture of methylheptanes	115 to 118
C_8H_{18}	*n*-Octane	125.8
C_9H_{20}	Mixture of methyloctanes	142 to 145
C_9H_{20}	*n*-Nonane	150.7
$C_{10}H_{22}$	Mixture of methylnonanes	165 to 168
$C_{10}H_{22}$	*n*-Decane	174.0
$C_{11}H_{24}$	Mixture of branched-chain undecanes	187 to 191
$C_{11}H_{24}$	*n*-Undecane	195.8
$C_{12}H_{26}$	Mixture of branched-chain dodecanes	207 to 211
$C_{12}H_{26}$	*n*-Dodecane	216.3
	Higher-boiling normal paraffins.	
	NAPHTHENIC	
C_5H_{10}	Cyclopentane	49.5
C_6H_{12}	Methylcyclopentane	71.9
C_6H_{12}	Cyclohexane	80.8
C_7H_{14}	Mixture of dimethylcyclopentanes	87.5 to 92
C_7H_{14}	Methylcyclohexane	100.9
C_8H_{16}	Mixture of dimethylcyclohexanes	120 to 124
	Higher-boiling naphthene concentrates.	
	AROMATIC	
C_6H_6	Benzene	80.1
C_7H_8	Toluene	110.6
C_8H_{10}	Ethylbenzene	136.2
C_8H_{10}	Mixture of *p*-xylene and *m*-xylene	138 to 140
C_8H_{10}	*o*-Xylene	144.4
C_9H_{12}	Isopropylbenzene	152.4
C_9H_{12}	*n*-Propylbenzene	159.5
C_9H_{12}	Mixture of 1-methyl- 3-ethylbenzene and 1 methyl- 4-ethylbenzene.	161 to 162
C_9H_{12}	Mixture of 1-methyl- 2-ethylbenzene and 1,3,5-trimethylbenzene.	164 to 165
C_9H_{12}	1,2,4-Trimethylbenzene	169.2
C_9H_{12}	1,2,3-Trimethylbenzene	176.1
$C_{10}H_{14}$	1,2,3,4-Tetramethylbenzene	204
$C_{10}H_{12}$	Tetrahydronaphthalene	207
$C_{10}H_8$	Naphthalene	218.0
$C_{11}H_{10}$	2-Methylnaphthalene	241.1
$C_{11}H_{10}$	1-Methylnaphthalene	244.8
	OLEFINIC [a]	
C_2H_4	Ethylene	−103.8
C_3H_6	Propylene	−47.7
C_4H_8	2-Methylpropene-1 (isobutene)	−7.0
C_4H_8	Butene-1	−6.4
C_4H_8	*trans*-Butene-2	1.0
C_4H_8	*cis*-butene-2	3.6
C_5H_{10}	3-Methylbutene-1	20.1
C_5H_{10}	Mixture of pentene-1 and 2-methylbutene-1	30 to 31
C_5H_{10}	Mixture of *cis*- and *trans*-pentene-2	35 to 37
C_5H_{10}	2-Methylbutene-2	38.4

[a] The olefinic hydrocarbons do not occur naturally in petroleum. They are produced mainly as products of the cracking process or by the specific reaction of dehydrogenating the corresponding paraffins.

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MICROSTRUCTURAL CHARACTERISTICS OF HIGH-PURITY ALLOYS OF IRON AND CARBON

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ABSTRACT

A study was made of the microstructural characteristics of slowly cooled high-purity alloys of iron and carbon of hypereutectoid composition and of the influence of certain impurities (oxygen, aluminum, and hydrogen) on the structures of the slowly cooled alloys. The alloys were prepared from 17 irons, varying in degrees of purity, by carburizing in a mixture of hydrogen and benzene vapor. The structure of the carburized irons of highest purity, free from aluminum and with a total of less than 0.009 percent of identifiable impurities, contained free ferrite in the hypereutectoid zone. If oxygen were responsible for this structural feature, then a minute amount was sufficient and as effective as larger quantities. Aluminum in excess of about 0.001 percent prevented the formation of free ferrite, and alumina was not the factor responsible for its formation in these alloys. The hydrogen dissolved in the irons during carburization had no detectable effect on the divorcement of ferrite. The experimental results indicate that a structure containing free ferrite is characteristic of very high purity alloys of iron and carbon of hypereutectoid composition that have been slowly cooled from the austenitic condition.

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I. INTRODUCTION

The existence of free ferrite adjacent to coalesced masses of cementite in the hypereutectoid zone of carburized specimens from certain heats of plain carbon steel was reported in 1922 by McQuaid and Ehn [1].[1] Steels showing this lack of perfection in the crystallization of pearlite after carburizing were designated as "abnormal." Such steels usually have relatively fine-grained austenite at the carburizing

[1] The figures in brackets refer to the literature references at the end of this paper.

temperature. In "normal" steels the austenitic grain size usually is relatively large, and, after slowly cooling from the carburizing temperature, the pearlite extends continuously to thin envelopes of cementite in the hypereutectoid zone. In McQuaid and Ehn's practice, the surfaces of the normal steels were completely hardened whereas the surfaces of the abnormal steels frequently contained soft spots after the quenching treatment. Abnormality was attributed to the presence of oxides, either in solution or as submicroscopic particles.

The choice of the words "normal" and "abnormal" to designate the differences in structure and hardenability of these carburizing steels appeared to be reasonable and suited to this specific use. The terms are clear to all when they are restricted to their original application, namely to plain carbon steels of the carburizing type, but confusion results when the terms are applied to other grades of alloys and steels. This nomenclature has been the concern of many metallurgists, as shown by the comments of Epstein and Rawdon [2], Davenport and Bain [3], and others. To eliminate confusion in the present report, the terms "normal" and "abnormal" will be retained only for reference to previous investigations. The structures produced in the slowly cooled alloys may be completely identified by representative micrographs and by naming all the constituents therein observed, such as free ferrite, massive cementite, or cementite and pearlite. The structure containing free ferrite (divorced ferrite) corresponds to that previously designated as abnormal.

The present study was made primarily to determine the influence of small amounts of impurities, particularly oxygen, aluminum, and hydrogen, in different irons, on the structures produced by oxygen-free carburizing followed by cooling slowly and at a constant rate through the transformation range.

II. PREVIOUS INVESTIGATIONS

McQuaid and Ehn's work with commercial steels was followed by numerous investigations carried out by carburizing both commercial steels and high-purity irons. A survey of some of the publications on this subject shows wide differences in opinion regarding the factors responsible for normality and abnormality. In 1928 Epstein and Rawdon [2] pointed out that many cases of abnormality in commercial steels seemed to be associated with the use of aluminum for deoxidizing, although abnormal steels could be produced in other ways. Nothing arose during their experiments to disprove Ehn's theory that abnormality was due ordinarily to the presence of oxides, perhaps dissolved but more probably undissolved. Several years later, Grossman [4] was of the opinion that abnormality was due to oxygen dissolved during carburization.

In 1932 Duftschmid and Houdremont [5] reported the presence of abnormal structures at the surfaces of electrolytic and carbonyl irons after carburizing. They contended that a prerequisite to abnormal structure was a rapid rate of crystallization, which occurred when the Ar_1 transformation took place at a very high temperature. The regions of highest purity were believed to be responsible for abnormality in carburized structures.

In 1932 Bain [6] reported the results of carburizing experiments with a variety of irons obtained from widely different sources. These irons developed abnormal structures in pack-carburizing and, with one exception, in oxygen-free carburizing. The exception was a hydrogen-treated iron which was almost entirely normal after carburizing in pure hydrogen and hydrocarbon. This iron contained 0.005 percent of carbon, 0.003 percent of sulfur, 0.028 percent of manganese, 0.004 percent of phosphorus, 0.0012 percent of silicon, 0.003 percent of oxygen, and 0.0001 percent of nitrogen. With other irons containing aluminum in excess of 0.4 percent, normal structures were produced by carburizing in hydrocarbon and abnormal structures were developed in the outer portion of the hypereutectoid zone by pack-carburizing. Bain at that time was of the opinion that pure oxygen-free iron (perhaps with hydrogen in solution) would be normal after oxygen-free carburizing, and that dissolved oxygen lowered the critical point (Ae_1) and at the same time provided the high reaction rate and high carbon-diffusivity responsible for abnormality. To secure a normal structure by the standard McQuaid-Ehn procedure required the presence of an element such as silicon or aluminum, which contributed to deep hardening, or at least materially reduced oxygen solubility. However, in 1934, Davenport and Bain [3] were of the opinion that carburized high-purity iron was abnormal.

In 1937 Brophy and Parker [7] reported the results of carburizing experiments with irons containing 0.001, 0.01, 0.1, and 1 percent of aluminum. Normal structures were produced in the cases of all the irons when carburized in a mixture of hydrogen and benzene vapor and abnormal structures when pack-carburized. They concluded that extremely small amounts of aluminum were sufficient to cause abnormality if oxygen was available. However, in the discussion McMullan pointed out that normality or abnormality in steel was a function of composition and cooling rate and that any of the common steels might have their apparent normality or abnormality greatly changed by varying the rate of cooling from the carburizing temperature. Aluminum by itself increases normality and grain size; but when both aluminum and oxygen were present in the right amounts, the steel was fine-grained, and hence might show an abnormal structure after carburizing. McQuaid was of the opinion that Brophy and Parker's results would have been more conclusive had a definite hypereutectoid case been obtained in both methods of carburizing and that the presence of hydrogen might have affected the final structure obtained by carburizing in hydrogen-benzene atmosphere.

In 1938, Derge, Kommel, and Mehl [8], reported the results of carburizing experiments with irons containing aluminum or silicon. All the irons had abnormal structures after pack-carburizing, and normal structures after carburizing in oxygen-free hydrocarbon vapor. This was interpreted as indicating that abnormality under these conditions was due to dissolved oxygen obtained from the carburizing gas rather than to aluminum, Al_2O_3, or oxides already existing in the metal. In the discussion, McQuaid reported that his results indicated that steels carburized in hydrogen-hydrocarbon atmospheres were normal due to the presence of hydrogen and not to the absence of oxygen and that silicon, with or without the presence of aluminum, was an important factor that affected the results obtained after carburizing.

The present writer, in discussing the paper by Derge and coworkers, submitted micrographs showing that abnormal structures were obtained in specimens of iron (total identifiable impurities less than 0.031 percent) which were carburized to contents of 1.01, 1.14, and 1.21 percent of carbon, respectively, in oxygen-free hydrocarbon vapor, homogenized, and subsequently heated at 1,700° F in vacuo and slowly cooled in vacuo. Analyses of duplicate specimens indicated that there was no change in the oxygen content of the specimen (0.003 percent) during the carburizing treatment, and it was difficult to believe that oxygen could have been picked up anywhere in the cycle, to cause the abnormal structures. Derge, however, suggested that oxygen might have entered the specimen during the vacuum treatments and cited experiments by Wells in which abnormal structures never were obtained when specimens of comparable purity were carburized in pure, oxygen-free hydrocarbon mixtures. In his closure, Derge expressed the opinion that the whole question of abnormality, in general, is one of relative rates, which may be influenced by a great many factors.

A review of the literature shows that abnormality has been attributed to:

1. Oxides, perhaps dissolved but more probably undissolved.
2. Alumina.
3. Oxygen dissolved during carburization.
4. Oxygen in solid solution in the original material.
5. High Ar_1 temperature.
6. High purity.
7. High carbon diffusivity.
8. High transformation rate (rapid rate of recrystallization).
9. Fine grain size.
10. Aluminum in small amounts, if oxygen is present.

Normality has been attributed to:

1. Aluminum.
2. Hydrogen.
3. Silicon.
4. Coarse grain size.
5. Low transformation rate.
6. Alloying elements of the deep-hardening type.
7. Alloying elements which reduce oxygen solubility.

Obviously some of the items listed as influencing either normality or abnormality are interrelated. It is now generally recognized that normality and abnormality are functions of composition and cooling rate.

III. MATERIALS AND EXPERIMENTAL PROCEDURE

Specimens from 17 irons were used in the present investigation. These irons were prepared by different methods and some differences existed in the identifiable impurities, as listed in table 1. The irons of highest purity, designated 1, 2, 5, 6, 11, 15, and 18, respectively, were prepared by Thompson and Cleaves, as described in detail in a previous report [9]. The procedure consisted essentially in converting purified iron oxide to sponge iron, followed by melting in a vacuum, treating with hydrogen while molten, and solidifying in a vacuum.

TABLE 1.—*Impurities determined in the irons*

Iron No.	Element (percentage by weight)																	
	Mn	P	S	Si	Cr	Al	Cu	Ni	Co	Ca	Pb	Mg	Be	Ge	O_2 [2]	N_2	H_2	Total identifiable [3]
BEFORE CARBURIZING																		
1	ND [1]		0.002	0.001	ND	ND	<0.002	ND	ND	ND	ND	ND	<0.001	ND	0.002_2	0.000_1	0.000_1	$<0.009_2$
2	ND	<0.0005	.002	.001	ND	ND	<.002	ND	ND	ND	ND	ND	<.001	ND	$.001_2$	$.000_2$	$.000_2$	$<.008_4$
5	ND		.001	.003	ND	<0.001	<.002	ND	ND	ND	ND	ND	ND	ND	$.004_9$	$.000_2$	$.000_3$	$<.012_8$
6	ND		.001	.001	ND	ND	<.002	ND	ND	ND	ND	ND	<.001	ND	$.005_4$	$.000_2$	$.000_2$	$<.011_6$
11	ND		.001	ND	ND	ND	<.002	ND	ND	ND	ND	ND	ND	ND	$.005_4$	$.000_2$	$.000_1$	$<.009_7$
15	ND		.001	ND	ND	<.001	<.002	ND	ND	ND	ND	ND	ND	ND	$.002_8$	$.000_6$	$.000_2$	$<.008_4$
18	ND		.001	ND	ND	ND	<.002	ND	ND	ND	ND	ND	ND	ND	$.004_3$	$.000_1$	$.000_2$	$<.008_2$
J [4]	0.013	ND	$.000_2$	.003	.0005	ND	.013	0.006	ND	ND	ND	ND	ND	ND	.001	.003	$.000_5$	<.045
D [5]	.002	<.001	.004	.002	ND	ND	<.001	.007	.007	<.001	<.001	<.001	ND	ND	.003	$.000_6$	$.000_1$	<.031
AFTER CARBURIZING [6]																		
A [7]	<0.001			<0.001	0.002	ND	0.003	0.02	<0.01				ND		0.010 to .017		0.000_1	
B	<.001			<.001	.003	ND	.002	.02	<.01	ND	ND	ND	FT [1]	T [1]	$.007_5$	$.000_7$	$.000_3$	
C	<.001			<.001	<.001	.1	<.001	.06	<.01	ND	ND	ND	T	ND	$.002_1$	$.000_4$	$.000_2$	
E	<.001			.008	.003	.025	.003	.02	<.01	ND	ND	ND	FT	T	.002 to .005	$.000_3$	$.000_2$	
F [7]	<.001			<.001	<.001	.1 to .2	.001	.06	<.01	ND	ND	ND	T	ND	$.001_5$	ND		
G [7]	<.001			.015	.002	<.005	.002	.01	<.01	ND	ND	ND	FT	ND	$.001_6$	$.000_2$	$.000_2$	
H [7]	<.001			.004	.002	.3	.003	.01	<.01	ND	ND	ND	W [1]	ND	$.002_8$	$.000_3$	$.000_3$	
I [7]				.015	<.001	.2	.001	.01	<.01	ND	ND	ND	T	T	$.001_8$	ND	$.000_2$	

[1] ND means not detected; T means trace; FT means faint trace; W means weak.
[2] Includes surface oxygen.
[3] Carbon was not included.
[4] This iron also contained 0.004 percent of Mo and traces of V and Sn.
[5] Some of the values reported were obtained on specimen after carburizing.
[6] Values for Mn were obtained by chemical analysis on specimens that were not carburized. Values reported for all of the other elements except gases for the irons after carburizing were determined by spectrochemical analysis.
[7] Values for gases obtained on specimens that were not carburized.

Chemical analyses were made by H. A. Bright, J. L. Hague, and W. H. Jukkola; spectrochemical analyses by B. F. Scribner and H. R. Mullin; and vacuum fusion analyses by V. C. F. Holm.

Iron *J* was carbonyl iron subsequently treated with hydrogen. The remaining irons were prepared from different lots of electrolytic iron melted and solidified in a vacuum. In some of the latter melts, a small amount of carbon was added to deoxidize the iron, and to iron *I* sufficient carbon was added (in the form of an iron-carbon alloy) to produce a carbon content of 1.12 percent in the final product.

The impurities, other than carbon, that were identified by spectrochemical and chemical methods, and the gas content, as determined by the vacuum-fusion method, are listed in table 1. The arc spectrum for each iron, except *A*, was examined for the sensitive lines of Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cb, Cd, Co, Cr, Cu, Ga, Ge, Hf, Hg, In, Ir, Mg, Mn, Mo, Na, Ni, Os, Pb, Pd, Pt, Re, Rh, Ru, Sb, Si, Sn, Ta, Th, Ti, U, V, W, Zn, and Zr. The sensitive lines of Ce, K, Li, Sc, Sr, Tl, and Y were sought in the spectra of all the irons of highest purity and of iron *D*. The elements identified, other than iron and carbon, are given in the table. The aluminum which is present as an impurity in some of the irons was obtained principally from the alumina crucibles used in preparing these particular heats.

The impurities were determined either on specimens from the original ingots or on specimens after the carburizing treatment, and the specimens used for carburizing were not necessarily taken from the same part of the ingot as the specimens used for analyses. However, in some instances, determinations of manganese, silicon, oxygen, nitrogen, and hydrogen were made on the same iron before and after carburizing, and the results, summarized in table 2, show that there was no appreciable change in these impurities incident to the carburizing treatment.

TABLE 2.—*Impurities determined in irons before and after carburizing in a mixture of hydrogen and benzene vapor*

Iron No.	Condition [1]	Impurities (percentage by weight)				
		Mn	Si	O_2	N_2	H_2
2	Before carburizing	ND [2]	0.001	0.001$_3$	0.000$_2$	0.000$_2$
2	After carburizing	ND	ND	.002$_8$	.000$_2$	.000$_3$
5	Before carburizing	ND	.003	.004$_9$	.000$_2$	.000$_2$
5	After carburizing	ND	<.001			
15	Before carburizing	ND	ND	.002$_8$	.000$_8$	.000$_2$
15	After carburizing	ND	ND			
B	Before carburizing			.007$_3$	.000$_7$	.000$_3$
B	After carburizing			.007$_2$	.000$_7$	.000$_3$
B	do		<.001	.007$_3$	.000$_7$	.000$_2$
C	Before carburizing			.003	.000$_2$	.000$_2$
C	After carburizing		<.001	.002$_1$	.000$_4$	.000$_2$
D	Before carburizing	.002	.002	.004$_1$	.000$_3$	.000$_2$
D	After carburizing		<.001	.003$_2$	.000$_5$	.000$_2$
E	Before carburizing			.003$_8$	.000$_3$	.000$_2$
E	After carburizing		.008	.004$_1$	.000$_8$	.000$_3$
E	do			.002	ND	.000$_2$
E	do			.002$_2$	.000$_2$	.000$_2$
E	do			.005	.000$_3$	.000$_2$

[1] For each iron, the different specimens were not always from the same part of the original ingot.
[2] ND means not detected.

Specimens of the different irons, usually between 0.04 and 0.05 in. in thickness, were carburized at about 1,700° F for 3 hours in

a mixture of hydrogen and benzene vapor, followed by cooling in this atmosphere through the Ar_1 transformation at approximately 4° F/min. Time in the carburizing temperature range was sufficient to produce a hypereutectoid zone throughout the cross section of the specimen. The benzene used was thiophene-free and conformed to American Chemical Society specifications. The hydrogen was purified and dried by passing in succession through Ascarite, silica gel heated to 1,300 to 1,350° F, magnesium perchlorate, and phosphorus pentoxide. The dry hydrogen next passed through the benzene and then into the quartz tube, where the specimens were suspended vertically by a wire of high-purity iron or iron-carbon alloy. The hydrogen finally passed into the air at the exit end of the train. The quartz tube was contained within an electrically heated furnace. The hot junction of a Chromel-Alumel thermocouple was located outside of the carburizing chamber, between the quartz tube and the furnace tube, in the uniformly heated zone.

The gas flow was not accurately determined or maintained constant during the carburization of the various irons, but the average flow was believed to be about 100 ml/min. The vapor not used in carburizing was burned at the exit end of the train.

The specimens were cleaned by washing in xylene or benzene and were then suspended in the cold zone of the quartz tube near the outlet. The tube was thoroughly flushed with the mixture of hydrogen and benzene vapor before lowering the specimens from the cold zone to the uniformly hot or carburizing zone.

Measurements of the austenitic grain size of the carburized specimens were made by the method described by Jeffries [10] and by comparison with the grain-size chart of the American Society for Testing Materials [11].

IV. STRUCTURES OF THE CARBURIZED IRONS

The structures obtained by carburizing the various irons at 1,700 °F for 3 hours in the hydrogen-benzene atmosphere followed by cooling in this atmosphere through the Ar_1 transformation at 4° F/min are summarized in table 3 and figures 1 to 6.

It is well known that the rate of cooling through the thermal critical range influences the divorcement of ferrite in hypereutectoid steels. Rejection of free ferrite could be entirely prevented in the present iron-carbon alloys by cooling at sufficiently high rates from the temperature used in carburizing. As pointed out by Davenport and Bain [3], cooling rates of 4° to 5° F/min are considered satisfactory for obtaining consistent results in normality studies. Obviously, the relative amounts of free ferrite in the alloys might have been different had the cooling rates differed widely from that used.

The impurities present in alloys of iron and carbon may be without effect or may act either to increase or to decrease the transformation rate of austenite or the diffusivity of carbon in the temperature range of the Ar_1 transformation and accordingly influence the divorcement of ferrite in the final structures. Since in this study it was difficult to isolate the effect of an individual impurity, it must be borne in mind that the structure produced in any alloy may have resulted from the combined effects of all of its impurities.

TABLE 3.—*Structure and austenitic grain size of iron-carbon alloys*

[Specimens were carburized at 1,700° F[3] for 3 hours in a hydrogen-benzene atmosphere and cooled in this atmosphere through the Ar_1 transformation at 4° F/min]

Iron				Treatment with hydrogen after solidification	Impurities (percentage by weight)					Austenitic grain size		Structure and remarks
No.	Preparation				Al	O_2[2]	N_2	H_2	Total identified	Average number of grains per square inch at ×100	ASTM No.	
	Base	Deoxidizer	Fabrication									
1	Sponge	Hydrogen	As cast	None	ND[1]	0.002	0.0001	0.0001	<0.010	0.5	0	Free ferrite, massive cementite network, and pearlite, as shown in fig. 1 (*A*).
2	do	do	do	do	ND	.001	.0002	.0002	<.009	1.0	1	Free ferrite, massive cementite network, and pearlite, as shown in fig. 6 (*A* and *B*).
5	do	do	do	do	<0.001	.005	.0092	.0002	<.013	0.2	−1	Very small amount of free ferrite, cementite network, and pearlite as shown in fig 1 (*D*). Mixed size with some very large austenitic grains.
6	do	do	do	do	ND	.005	.0002	.0002	<.012	0.8	1	Free ferrite, massive cementite network, and pearlite, as shown in fig. 5 (*A*).
11	do	do	do	2,000° F for 1 hour and water quenched.	ND	.005	.0002	.0001	<.010	1.6	2	Free ferrite, massive cementite network, and pearlite, as shown in fig. 5 (*B*).
15	do	do	Hot and cold worked.	None	<0.001	.003	.0006	.0002	<.009	0.6	0	Free ferrite, massive cementite network, and pearlite, as shown in fig. 1 (*C*). Mixed size austenitic grains.
18	do	do	As cast	do	ND	.004	.0001	.0002	<.009	0.6	0	Free ferrite, massive cementite network, and pearlite, as shown in fig. 1 (*B*).
A	Electrolytic	None	do	do	ND	.010 to .017		.0001		1.1	1	Free ferrite, massive cementite network, and pearlite, as shown in fig. 5 (*C*). Some elongated austenitic grains.
A	do	do	do	2,000° F for 1 hour and water quenched.						1.0	1	Free ferrite, massive cementite network, and pearlite, as shown in fig. 5 (*D*).
B	do	do	do	None	ND	.007	.0007	.0003		0.9	1	Free ferrite, massive cementite network, and pearlite, as shown in fig. 2 (*B*). Elongated austenitic grains.
C	do	do	do	do	0.1	.002	.0004	.0002		2.6	2	Cementite network and pearlite, as shown in fig. 3 (*A*).
D	do	do	Hot and cold worked.	do	ND	.003	.0005	.0001	<.031	5.6	3	Free ferrite, cementite network, and pearlite, as shown in fig. 2 (*A*).
E	do	Carbon	As cast	do	0.025	.004	.0003	.0002		5.0	3	Cementite network and pearlite with a very small amount of free ferrite in some areas, as shown in fig. 2 (*D*). Mixed size austenitic grains.
F	do	do	do	do	.1 to .2	.001	ND			6.5	4	Cementite network and pearlite, as shown in fig.

F	do	do	do	do	.1 to .2	.001	ND			6.5	4	Cementite network and pearlite, as shown in fig. 3 (*B*). Marked difference in austenitic grain size. Some No. −2 grains.
G	do	do	Hot- and cold-worked.	do	<0.005	.002	.0002	.0002		0.9	1	Cementite network and pearlite, as shown in in fig. 2 (*C*). Marked difference in austenitic grain size. Some No. −2 grains.
H	do	do	As cast	do	0.3	.003	.0003	.0003		9.5	4	Cementite network and pearlite, as shown in fig. 3 (*C*).
H	do	do	do	2,000° F for 1 hour and water quenched.						7.6	4	Cementite network and pearlite similar to fig. 3 (*C*). Some elongated austenitic grains.
H	do	do	Hot- and cold-worked.	do						2.8	2	Cementite network and pearlite similar to fig. 3 (*C*).
H	do	do	do	None		.002	.0004	.0002		1.0	1	Cementite network and pearlite, as shown in fig. 3 (*D*). Some elongated austenitic grains.
I	do	do	As cast (1.12% C).	do	0.2	.002	ND	.0002		2.1	2	Cementite network and pearlite, as shown in fig. 4 (*D*). Mixed size austenitic grains.
J	Carbonyl	Not melted	Hot-worked	Treated. Exact treatment unknown.	ND	.001	.003	.0005	<.045	0.3	−1	Small amount of free ferrite, cementite, and pearlite. Cementite principally in plates. Only a few austenitic grains delineated by cementite.

[1] ND means not detected.
[2] Includes surface oxygen.

1. INFLUENCE OF IMPURITIES

(a) OXYGEN

The aluminum-free irons of highest purity (total identifiable impurities less than 0.012 percent), with oxygen content ranging from about 0.001 to 0.005 percent, had appreciable amounts of free ferrite in the final structures, as illustrated in figure 1, *A* and *B*. However, this difference in oxygen content had no detectable effect on the amount of free ferrite. If oxygen, either in the form of oxides or more probably in solution, was responsible for this structural condition then a minute amount of the element evidently was sufficient and as effective as larger amounts. Furthermore, in the aluminum-free irons of intermediate purity (*B* and *D*, table 1) more free ferrite was obtained in the carburized structure of the iron containing 0.003 percent of oxygen (fig. 2, *A*) than in the iron containing 0.007 percent of oxygen (fig. 2, *B*).

Oxygen contents of about 0.001 percent as determined by the vacuum-fusion method represent a close approximation to oxygen-free material. The vacuum-fusion method determines the total oxygen content, that is, the film of oxides on the surface of the specimen as well as the oxygen in the interior of the specimen. Evidence has been obtained indicating that the surface film on certain specimens amounts to about 0.001 percent. For these reasons vacuum-fusion determinations of less than 0.001 percent of oxygen are seldom obtained, even for the most carefully deoxidized material

The oxygen content, from 0.001 to 0.007 percent, could not be correlated with the divorcement of free ferrite in the final structure of these alloys, which were free from aluminum but some of which contained appreciable amounts of other impurities. A structure containing free ferrite is characteristic of very high-purity alloys of iron and carbon of hypereutectoid composition, with very low oxygen content, that have been cooled slowly from the austenitic condition through the thermal critical range.

(b) ALUMINUM AND ALUMINA

The carburized structures of all of the irons which were free from aluminum contained free ferrite. All of the irons which contained more than 0.001 percent of aluminum had carburized structures that were practically free from ferrite.

Of the two carburized irons that contained a trace of aluminum (less than 0.001 percent), number 15 had an appreciable amount of free ferrite in the hypereutectoid zone (fig. 1, *C*) and number 5 had only a small amount of free ferrite (fig. 1, *D*). The values obtained by analyses indicated that sufficient oxygen was available in each of these carburized irons (0.003 and 0.005 percent of oxygen, respectively) to combine with all of the aluminum to form alumina, provided the greater portion of the oxygen reacted in this manner. Thus it appears probable that both alloys were free from metallic aluminum and each had approximately the same amount of alumina. Evidently the differences in amounts of free ferrite in the final structures were not due to differences in metallic aluminum or alumina. A noteworthy feature is that, when a trace of aluminum was present, the carburized specimens from the iron with lower oxygen content had the greater

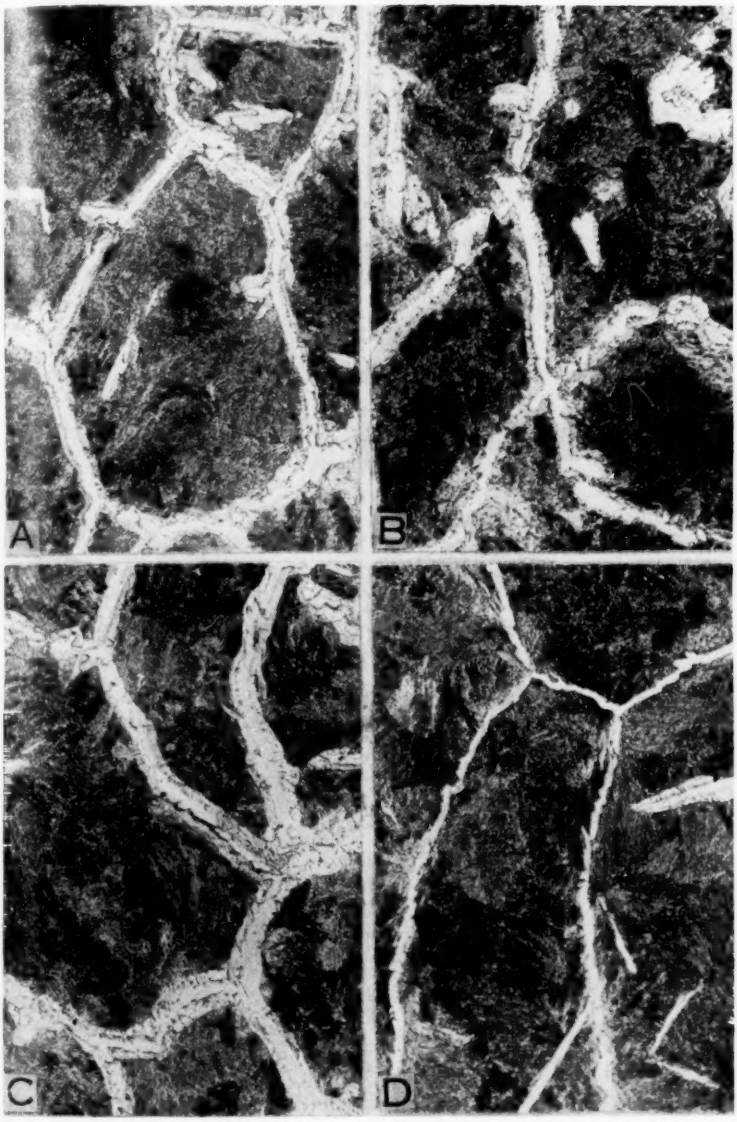

FIGURE 1.—*Structure of high-purity irons after carburizing.*

A, Iron 1, with 0.002 percent of O_2, no Al, and <0.010 percent of total identifiable impurities; *B*, Iron 18, with 0.004 percent of O_2, no Al, and <0.009 percent of total identifiable impurities; *C*, Iron 15, with 0.003 percent of O_2, <0.001 percent of Al, and <0.009 percent of total identifiable impurities, *D*, Iron 5, with 0.005 percent of O_2, <0.001 percent of Al, and <0.013 percent of total identifiable impurities. Etched with 1-percent nital. ×100.

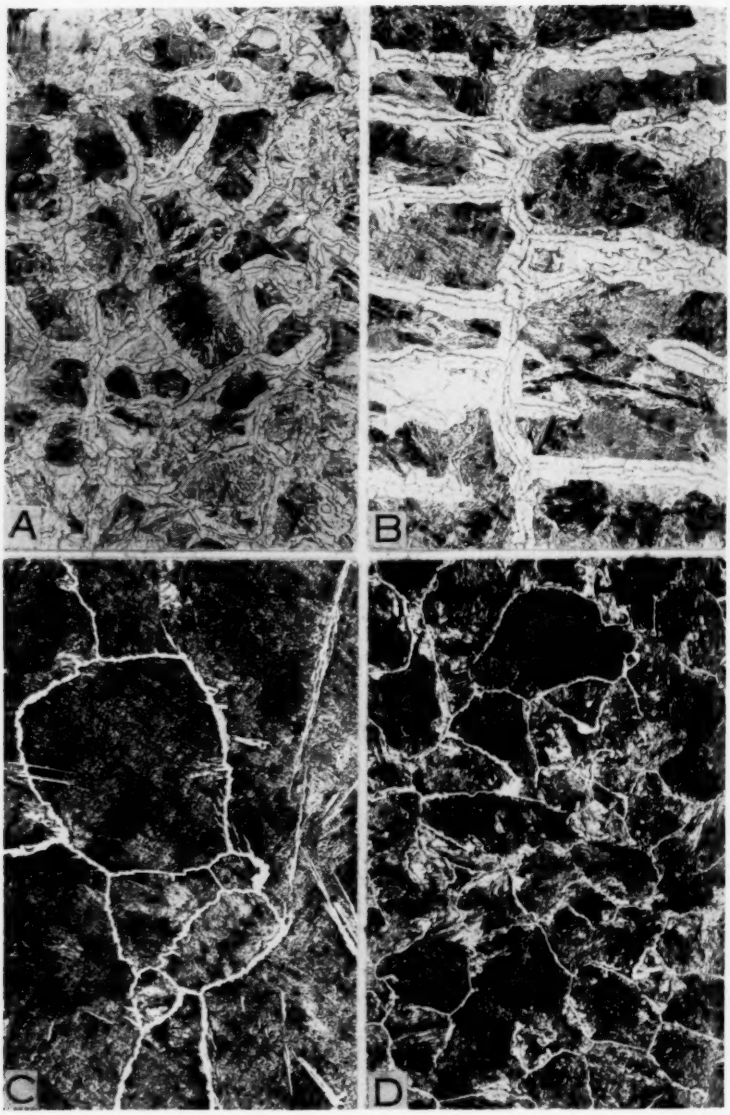

FIGURE 2.—*Structure of intermediate-purity irons after carburizing.*

A, Iron *D*, with 0.003 percent of O_2 and no Al; *B*, Iron *B*, with 0.007 percent of O_2 and no Al; *C*, Iron *G*, with 0.002 percent of O_2 and <0.005 percent of Al; *D*, Iron *E*, with 0.004 percent of O_2 and 0.025 percent of Al. Etched with 1-percent nital. ×100. Irons *D* and *G* were hot- and cold-worked prior to carburizing, whereas irons *B* and *E* were as cast.

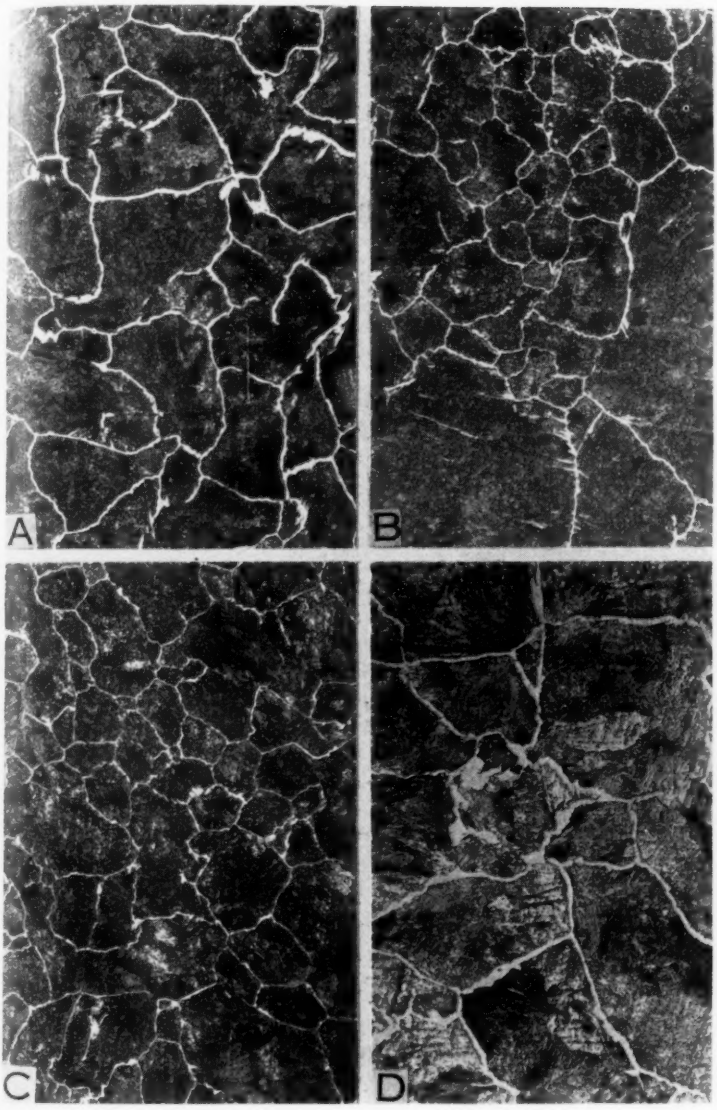

FIGURE 3.—*Structure of irons containing aluminum after carburizing.*

A, Iron *C*, with 0.002 percent of O_2 and 0.1 percent of Al; initial structure as cast; *B*, Iron *F*, with 0.001 percent of O_2 and 0.1 to 0.2 percent of Al; initial structure as cast; *C*, Iron *H*, with 0.003 percent of O_2 and 0.3 percent of Al; initial structure as cast; *D*, Iron *H*, with 0.002 percent of O_2 and 0.3 percent of Al; initial structure as hot- and cold-worked. Etched with 1-percent nital. ×100.

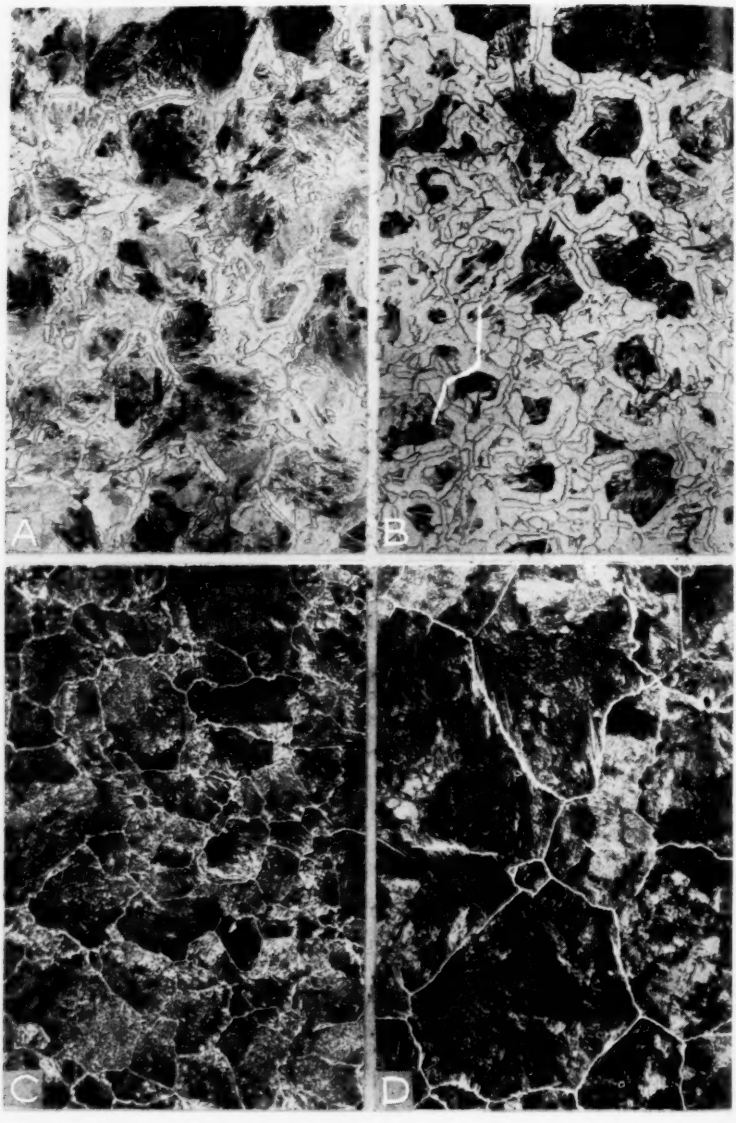

FIGURE 4.—*Structure of iron-carbon alloys after annealing in vacuo or as carburized.*

A, 1.01 percent of C, prepared from iron *D*, after annealing in vacuo; *B*, 1.21 percent of C, prepared from iron *D*, after annealing in vacuo; *C*, 1.12 percent of C, prepared from iron-carbon alloy *I*, after annealing in vacuo; *D*, Iron-carbon alloy *I*, after carburizing. The annealing treatment consisted of heating in vacuo at 1,700° F for 1 hour and cooling through the Ar_1 transformation at 4° F/min. Etched with 1-percent nital. ×100.

amount of free ferrite, a fact which is contrary to the prevailing opinion.

The carburized structure of iron G with less than 0.005 percent of aluminum (0.015 percent of silicon) was essentially free from ferrite (fig. 2 C) and that of iron *E* with 0.025 percent of aluminum (0.008 percent of silicon) contained only a small amount of free ferrite in some areas (fig. 2, *D*). The relatively high silicon content was perhaps a factor in preventing the formation of ferrite in these alloys, especially in the one with the lower aluminum content. However, the carburized structures (fig. 3) of the irons with aluminum ranging from 0.1 to 0.3 percent (silicon from trace to 0.004 percent) contained no free ferrite. It should be pointed out that the two alloys with a trace of silicon also contained relatively high nickel (0.06 percent), but the amount of nickel in the other alloy (0.01 percent) was only slightly higher than that of the aluminum-free alloy of figure 2 (*A*) (0.007 percent of nickel), which contained a large amount of free ferrite.

The values for oxygen ranged from approximately 0.001 to 0.005 percent in the alloys containing aluminum and, as already stated, from 0.001 to 0.007 percent in the aluminum-free alloys which contained appreciable amounts of free ferrite in the final structures. In the alloys of low aluminum content, the aluminum should be present almost entirely as the oxide (alumina) and, while the alloys of high aluminum content also contained alumina, the greater portion should be present as the metal. It is generally believed that alumina increases the decomposition rate of austenite to ferrite and carbide by acting as centers for transformation, whereas metallic aluminum enters into solid solution in the austenite and thereby decreases its transformation rate. The present results show that aluminum in excess of about 0.001 percent inhibits the divorcement of ferrite and that alumina was not the factor responsible for its formation in these hypereutectoid alloys.

(c) HYDROGEN

Carburized and homogenized specimens prepared from iron *D* [12] containing 1.01 and 1.21 percent of carbon, were annealed in an atmosphere of dry hydrogen at 1,700° F for 1 hour and cooled through the Ar_1 transformation at 4° F/min. Other specimens of the same alloys, with the same initial structure of fine pearlite, were given a similar thermal treatment in vacuo. During the time of cooling from the annealing temperature through the transformation range, the former specimens were saturated with hydrogen whereas the specimens annealed in vacuo were relatively free from hydrogen. The surfaces of the specimens annealed in hydrogen were decarburized, but a hypereutectoid zone was retained in the center of some of the specimens. The hypereutectoid zones contained free ferrite and had a structure similar to that of the vacuum-annealed specimens shown in figure 4 (*A* and *B*). Furthermore, the amount of free ferrite obtained in the latter specimens was of the same order of magnitude as that of specimens of the same iron *D* carburized at 1,700° F in a mixture of hydrogen and benzene vapor and cooled at a similar rate directly from the carburizing temperature in an atmosphere containing hydrogen (see fig. 2, *A*). Thus this structural feature of free ferrite in slowly cooled hypereutectoid alloys was produced with equal facility either

by cooling directly from the carburizing temperature or by subsequently annealing in vacuo or in hydrogen.

The structure of alloy *I* (containing 1.12 percent of carbon as cast) after the annealing treatment in vacuo (fig. 4, *C*), and the structure of the same alloy after the carburizing treatment (fig. 4, *D*), consisted of cementite network and pearlite. A marked increase in austenitic grain size resulted from the carburizing treatment in which the specimen was held at 1,700° F for 3 hours in an atmosphere containing hydrogen. Free ferrite was not produced in the structure of alloy *I* either by annealing in hydrogen or in vacuo.

Specimens from several different irons were treated in dry hydrogen at approximately 2,000° F and quenched in water (table 3). Although the time at high temperature was sufficient for the hydrogen to diffuse throughout the cross section of the specimens, it was not sufficient to cause an appreciable reduction in the oxygen content, as shown by determinations made on similarly treated specimens. The hydrogen content of the specimens immediately after quenching varied; the maximum value was about 0.0005 percent. The hydrogen was extracted by heating at about 1,500° F for 6 to 15 minutes [13] before carburizing in the hydrogen-benzene atmosphere in the usual manner. The structures produced by carburizing untreated high-purity iron 6 and hydrogen-treated iron 11 of equivalent purity, and untreated and hydrogen-treated iron *A*, are shown in figure 5. Free ferrite was present in all of these specimens, and no difference in the amount of free ferrite was obtained as a result of the hydrogen treatment (compare fig. 5, *A*, with fig. 5, *B*, and fig. 5, *C*, with fig. 5, *D*).

These results show that hydrogen had no influence on the final structures obtained in the slowly cooled alloys.

2. INFLUENCE OF TOTAL IMPURITIES

The results summarized in table 3 show, in general, that the irons of highest purity (total identifiable impurities less than about 0.010 percent) had structures containing free ferrite in the hypereutectoid zone after carburizing and slowly cooling in an oxygen-free atmosphere. However, the carburized structure of iron 5 (total impurities less than 0.013 percent) contained only a relatively small amount of free ferrite (fig. 1, *D*) as compared with the carburized structure (fig. 1, *C*) of iron 15 (total impurities less than 0.009 percent). Since specimens from these two irons were carburized at the same time, the different amounts of free ferrite in the final structures must be attributed to differences in composition and not to the carburizing conditions. As shown in table 1, the identifiable impurities for the original ingots of irons 5 and 15 differed mainly in silicon (0.003 percent and nil, respectively) and oxygen (0.005 and 0.003 percent, respectively), but these differences are of doubtful significance because of the uncertainty of analytical determinations of this order of magnitude. Spectrochemical analysis showed that no definite change in the amounts of impurities occurred during the carburizing treatment, and it has already been shown that the carburizing treatment did not change appreciably the amounts of the gaseous elements. It is difficult, therefore, to explain satisfactorily the observed differences in the percentages of free ferrite on the basis of such minute variations in these detectable impurities.

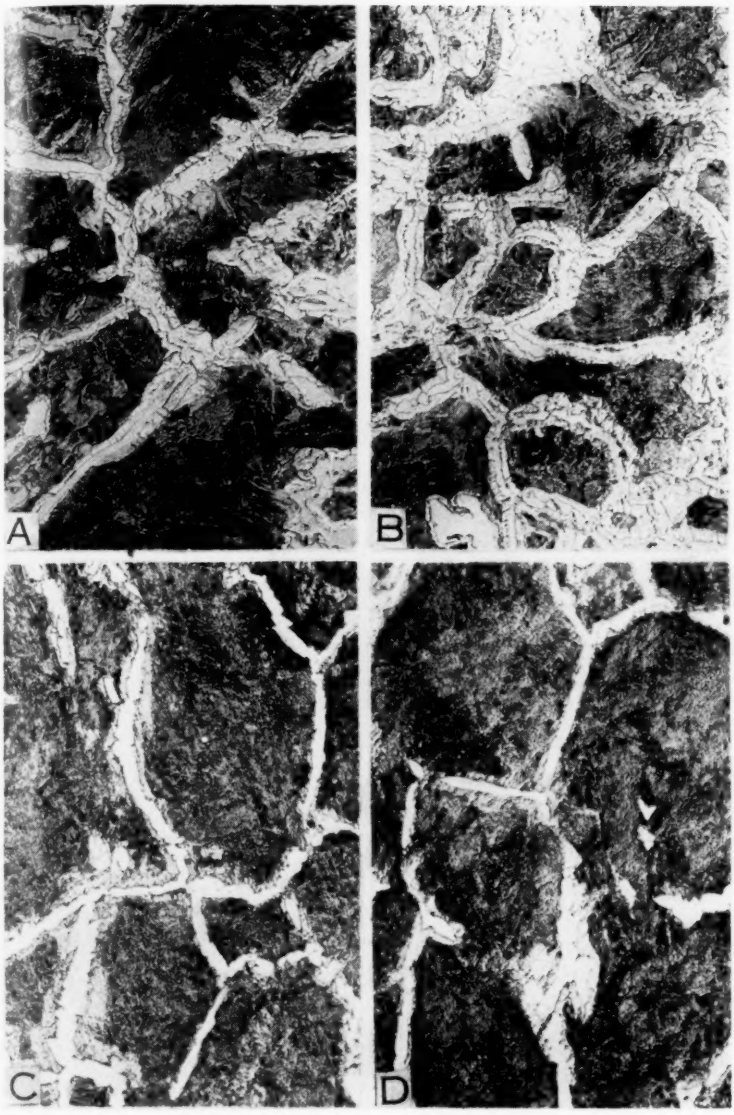

FIGURE 5.—*Structure of irons after carburizing.*

A, Iron 6, not treated with hydrogen before carburizing; *B*, Iron 11, treated with hydrogen before carburizing; *C*, Iron *A*, not treated with hydrogen before carburizing; *D*, Iron *A*, treated with hydrogen before carburizing. The hydrogen treatment consisted of heating specimens of the irons as cast in dry hydrogen at 2,000° F for 1 hour and quenching in water. Etched with 1-percent nital. ×100.

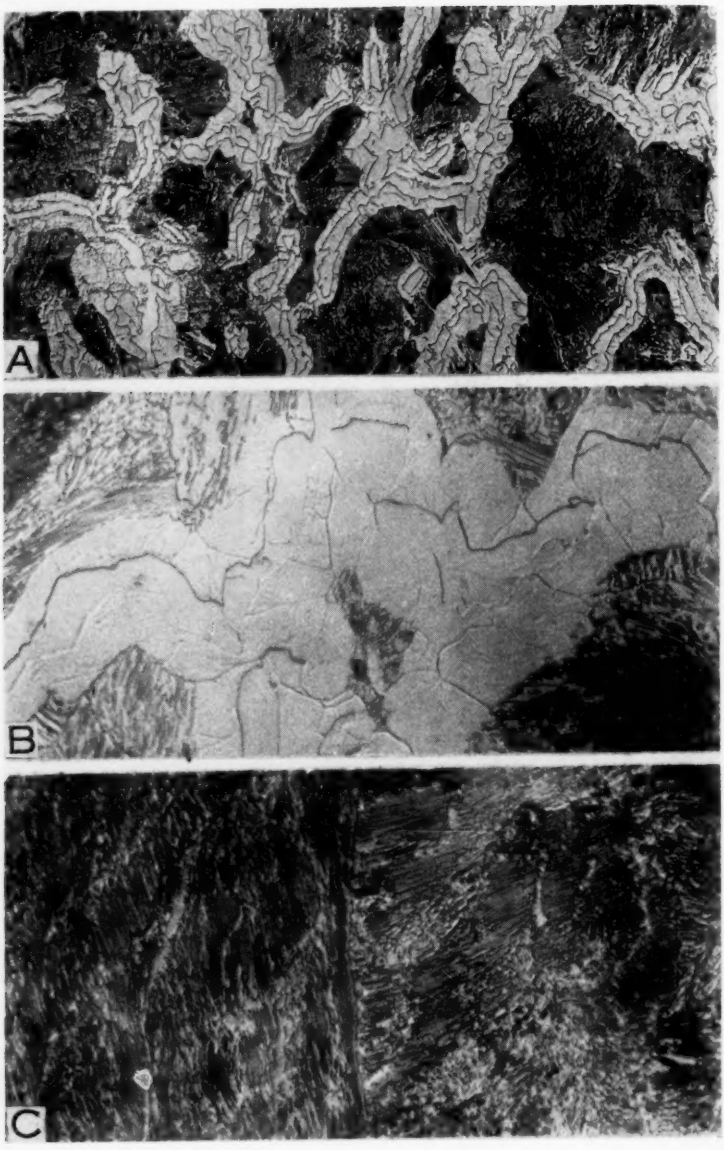

FIGURE 6.—*Structure of the hypereutectoid and eutectoid zones produced in high-purity iron by carburizing.*

A, Iron 2, hypereutectoid zone, ×100. *B*, Iron 2, hypereutectoid zone, ×500. *C*, Iron 2, eutectoid zone, ×500. Etched with 1-percent nital.

Different amounts of free ferrite were obtained in the carburized structures of the irons of intermediate purity (0.03 to 0.05 percent of identifiable impurities), whereas the carburized structures were essentially free from ferrite for the irons of relatively low purity (appreciable amounts of aluminum).

These results show that the presence of free ferrite in the hypereutectoid zone of very high-purity alloys of iron and carbon is characteristic of the structures obtained after slowly cooling from the carburizing temperature. As the amounts of impurities are increased, the final structures obtained in the alloys by slowly cooling after carburizing may or may not contain free ferrite in the hypereutectoid zone, depending on whether the added impurities are of the type which increase or decrease the reaction rate of austenite or the diffusivity of carbon in the Ar_1 transformation range.

3. INFLUENCE OF OTHER FACTORS

(a) CARBON

Divorcement of ferrite was not observed in the eutectoid zone of any of the carburized specimens. As illustrated in figure 6, the presence of a proeutectoid constituent (carbide in this case) in austenite appears to be essential for the production of free ferrite in the structure of iron-carbon alloys cooled at 4° F/min through the thermal critical range. With slowly cooled alloys of eutectoid content, the austenite transformed entirely to pearlite (fig. 6, *C*). However, variations may exist in the carbon content in excess of the eutectoid without appreciably influencing the amount of free ferrite in the final structure of the slowly cooled alloys, as illustrated in figure 4 (*A* and *B*).

(b) ANNEALING TREATMENT

It has already been pointed out that carburized and homogenized specimens containing 1.01 and 1.21 percent of carbon (prepared from iron *D*) and subsequently annealed in a vacuum had approximately the same amount of free ferrite in the final structures (fig. 4, *A* and *B*) as specimens of the same iron carburized to produce a hypereutectoid alloy in the hydrogen-benzene atmosphere, followed by cooling in this atmosphere at the same rate (fig. 2, *A*).

No increase in oxygen content of the iron-carbon alloys occurred during the time of annealing in vacuo, as shown by the values obtained for oxygen as follows:

Iron or alloy designation	Condition	Oxygen (percentage by weight)
D	As carburized	0.003
D	As carburized and annealed	.003
I	As cast (1.12 percent of C)	.002
I	As cast and annealed	.001

The structural feature consisting of free ferrite in slowly cooled hypereutectoid alloys was produced with equal facility either by cooling directly from the carburizing temperature or by subsequently annealing in vacuo.

(c) INITIAL STRUCTURE

For most of the irons, the structure prior to carburizing was that of the cast metal. In several irons, however, the initial structure was that obtained by hot- and cold-working the cast metal, and the carbonyl iron had been hot-worked after sintering. As shown by the results summarized in table 3, figure 2 (*A* and *B*), and figure 3 (*C* and *D*), the amount of free ferrite obtained in the carburized structure was independent of the initial structure of the irons, whether as cast or as hot- and cold-worked.

4. INFLUENCE OF AUSTENITIC GRAIN SIZE

In a previous report [14] it was shown that the austenitic grain size affected the rate of decomposition of austenite in the *Ar'* temperature range of high-purity alloys of iron and carbon. The critical cooling rate progressively decreased (hardenability increased) as the austenitic grain size increased in alloys containing 0.80 to 0.85 percent of carbon. Davenport, Grange, and Hafsten [15] recently reported that with an increase in austenitic grain size the isothermal transformation of austenite in an SAE 4140 steel was retarded in the temperature levels (about 1,050° F and above) where soft lamellar structures form.

The grain sizes established in the various irons by carburizing at 1,700° F were determined, and the results are summarized in table 3. Variations existed in the average size of the austenitic grains, and in some cases grains of mixed sizes were obtained in the same alloy; but no correlation was found between austenitic grain size and divorcement of ferrite in the final structures of the slowly cooled hypereutectoid alloys. For example, two of the alloys which had structures free from ferrite had average American Society for Testing Materials grain number 4 (fig. 3, *C*) and grain number 1 (fig. 2, *C*, mixed-size grains containing some No. −2), whereas two other alloys with approximately the same average grain sizes had structures containing appreciable amounts of free ferrite (fig. 2, *A*, and 6, *A*, respectively). An interesting feature is the large austenitic grains and a structure containing free ferrite which was obtained in the alloys of highest purity, as shown in figure 1.

V. SUMMARY

Seventeen irons, varying in degrees of purity, were carburized in a mixture of hydrogen and benzene vapor at 1,700° F for 3 hours followed by cooling in this atmosphere through the transformation range at 4° F/min. The time at the carburizing temperature was sufficient to produce hypereutectoid zones in all of the irons.

The presence of free ferrite in the hypereutectoid zones is characteristic of the structures obtained in irons of very high purity under these conditions. As the amounts of impurities in the irons are increased, the final structures obtained after carburizing may or may not contain free ferrite in the hypereutectoid zone, depending upon whether the added impurities are of the type which increase or decrease the reaction rate of austenite or the diffusivity of carbon in the Ar_1 transformation range. Differences in oxygen content from about 0.001 to 0.007 percent could not be correlated with the amount of free ferrite in

the final structure of the alloys. Some of the irons represent a close approximation to oxygen-free material. The precise role of oxygen in the development of ferrite in the final structure is not entirely clear. However, if oxygen were responsible for this structural feature in the hypereutectoid alloys of highest purity, then a minute amount was sufficient and as effective as larger amounts.

The carburized structures of all the irons which were free from aluminum contained free ferrite. Aluminum in excess of about 0.001 percent inhibits the formation of free ferrite in the hypereutectoid zone, and alumina was not the factor responsible for its formation.

The presence of hydrogen in the carburizing and annealing atmospheres had no detectable effect on the formation of free ferrite.

Free ferrite was not detected in the eutectoid zone of any of the alloys. However, variations might exist in amounts of proeutectoid carbide without markedly affecting the formation of free ferrite on slowly cooling from the austenitic condition.

Free ferrite in hypereutectoid alloys was produced with equal facility either by cooling directly from the carburizing temperature or by annealing in vacuo. The initial structures of the irons, either as cast or after working, had no effect on the formation of free ferrite in the final structures.

The average grain size established in carburizing the various irons at 1,700° F ranged from American Society for Testing Materials grain number 4 to —1, but there existed no correlation between austenitic grain size and the formation of free ferrite. A noteworthy feature was the large austenitic grains of the alloys of highest purity which contained relatively large amounts of free ferrite.

Acknowledgment is made to H. E. Cleaves and J. G. Thompson for specimens of the highest-purity irons, and to V. C. F. Holm, for specimens of some of the irons and for the determinations of the gas content of the irons and alloys.

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PHOTOCHEMICAL REACTIONS IN SILK

By Henry A. Rutherford and Milton Harris [1]

ABSTRACT

The stability of silk to light appears to be in part influenced by the cations in the silk. The removal of cationic substances from silk by two independent methods results in practically identical decreases in the stability of the fibers to light; the introduction of cations by treatment with basic compounds appreciably increases the stability.

Methylation of the carboxylic acid groups in silk by treatment with diazomethane does not appreciably change the photochemical behavior of the fiber, whereas methylation of only a small percentage of the hydroxyl groups of the tyrosine results in a marked increase in photochemical stability. The results suggest that alteration of the tyrosine hydroxyl groups by reaction with various reagents is responsible for at least part of these stabilizing effects.

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I. INTRODUCTION

The results of numerous investigations have indicated that silk is probably more readily deteriorated by light than any other natural textile fiber. While the mechanism of the decomposition is not clear, it appears that the rate of decomposition may be considerably altered by pretreatment of the fiber with various chemical reagents. For example, Harris and Jessup [1, 2] [2] found that silk treated with dilute solutions of sodium hydroxide is more stable towards the action of light than untreated silk, whereas the stability of silk treated with dilute solutions of sulfuric acid is greatly decreased. These findings were later substantiated by investigations by Hall [3]. Bonicatti [4], on the other hand, claimed that pretreatment with alkalies has no protective effect, but he did observe the photochemical decomposition to be greatly accelerated by treatment of the fiber with dilute solutions of mineral acids. More recently, Weilenmann [5] showed that some

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[2] Figures in brackets indicate the literature references at the end of this paper.

aliphatic and aromatic amines exhibit a protective action, whereas compounds such as aliphatic amides and amino acids are not effective.

The efficacy of the basic compounds in inhibiting decomposition is of considerable interest, especially since it has recently been found by Gleysteen and Harris [6] that most of the base taken up by silk is bound by the hydroxyl groups of the amino acid tyrosine. It is now shown that the photochemical behavior of silk is associated with the behavior of the tyrosine and that alteration of the tyrosine hydroxyl groups by reaction with various reagents is responsible for at least part of the stabilizing effects noted in the past.

II. EXPERIMENTAL PROCEDURE

1. MATERIALS

The silk used in this work was a plain-woven cloth, commercially degummed, having 94 threads per inch. The cloth was first extracted with cold alcohol and with ether, and then washed in cold distilled water to remove the organic solvents. This silk is hereafter referred to as *degummed* silk.

The removal of cations from the silk was accomplished either by electrodialysis or by treatment with acid below pH 2.5. This material is hereafter referred to as *electrodialyzed* silk or *acid-treated* silk, according to the process by which it was prepared. Purification by electrodialysis has the advantage of eliminating possible secondary reactions which may be produced by treatment of the fibers with acidic solutions.

Methylated samples were prepared using diazomethane according to the procedure recently described by Rutherford, Patterson, and Harris [7]. The acidic groups of degummed and of electrodialyzed silk have different rates of reaction with diazomethane, and it was possible to methylate an approximate, predetermined fraction of the carboxylic acid and tyrosine hydroxyl groups. The extent of methylation of each of these groups was directly determined by the procedure previously described [7]. The decrease in tyrosine content is a measure of the hydroxyl groups methylated. The difference between the total methoxyl content and the methoxyl content accounted for by the decrease in tyrosine content is a measure of the carboxyl groups esterified. Samples [3] I and II were treated for ¼ and for 1½ hours, respectively, in an ethereal solution of diazomethane but were not electrodialyzed or wet out with alcohol before the treatment; sample III was first soaked in 75-percent alcohol and then treated for ¼ hour with diazomethane. All of these samples were then electrodialyzed to remove cationic substances. Samples IV and V were electrodialyzed, wet with alcohol, and then treated for ¼ and for 16 hours, respectively, with diazomethane. With the exception of sample V, which lost about 15 percent in strength, the methylation process had little effect on the strength of the fabric

2. METHODS

The general procedure used in the preparation of the samples for irradiation was as follows: Pieces of the cloth were soaked for 30 minutes in a solution of a given reagent, the ratio of silk to solution

[3] See table 3, p. 86.

being 1 to 100. The greater part of the solution was removed by centrifuging, the cloth being suspended on a wire rack in a centrifuge tube so that practically complete separation of the sample and solution was obtained. When the same piece of cloth was given one or more additional treatments, this same procedure was followed for each treatment. The silk cloth was finally air-dried and then cut into warpwise strips 1¼ inches wide, which were then raveled down to 94 warp threads suitable for the breaking-strength test. The samples were then exposed to the light for 20 hours. To determine the effect of light alone, a second group of samples, treated and prepared in the same way, was kept for 20 hours in the dark under conditions of temperature and humidity approximating those encountered during the light exposure.

The decrease in breaking strength of the cloth was used as a measure of the deterioration of the silk. The tensile strengths were determined on a pendulum-type machine, the samples being conditioned and tested in a room maintained at a temperature of 21° C and a relative humidity of 65 percent.

The source of light was the Eveready accelerated testing unit, type C3A, equipped with Eveready Sunshine carbons and Corex D ultraviolet-transmitting glass. The exposures were made for 20 hours at a temperature of 55° to 60° C.[4]

The cationic contents of the various samples were determined quantitatively by the electrodialytic method of Sookne, Fugitt, and Steinhardt [8]. Tyrosine was determined by Lugg's method after an alkaline hydrolysis [9], and methoxyl content by the method of Viebŏck and Brecker [10].

III. RESULTS AND DISCUSSION

1. EFFECT OF REMOVAL OF CATIONS ON THE RATE OF DETERIORATION

It was observed in preliminary experiments that pretreatment of the silk with practically any basic compound has the effect of reducing the rate of deterioration. This suggested the possibility that the increased stability produced by these substances might result from combination of cations with the acidic groups of the fibers, which are of two types—namely, carboxylic acid and tyrosine hydroxyl groups—and conversely, that the decreased stability observed upon treatment with dilute mineral acids might be due in part to removal of cationic substances from these groups. On the basis of these considerations, it appeared advisable to study the behavior of silk freed from these substances by electrodialysis and by treatment with acid.

The results are shown in table 1. Electrodialysis of the silk resulted in lowering the cation content from 0.13 to 0.01 milliequivalent per gram and at the same time appreciably decreased the stability of the silk to light. Similar results were obtained when the samples were pretreated with solutions of hydrochloric, sulfuric, or acetic acids at pH 2.4.[5] Thorough washing of the acid-treated samples with distilled water prior to exposure did not alter the stability to light, which indicates that the decrease in stability results from removal of cations

[4] This was measured by a mercury thermometer, the bulb of which was covered by a piece of the silk, and placed the same distance from the light as the samples.

[5] The choice of this pH was based on the titration studies of Gleysteen and Harris [6] in which it was shown that removal of cations from silk becomes appreciable below about pH 3.

and is not caused by the presence of free acid in the exposed samples. It was found that none of the silk samples kept in the dark exhibited any deterioration, showing that the deterioration of the irradiated silk was not thermal, but photochemical.

TABLE 1.—*Effect of electrodialysis and of treatment with dilute acids on the deterioration of silk cloth*

Sample	Treatment	Cation content	Loss in breaking strength on exposure to light
		M eq/gm	%
Degummed	None	0.13	32
Electrodialyzed	do	.01	41
Degummed	H_2SO_4 (pH 2.4)	.004	43
Do	H_2SO_4, followed by water wash	.005	41
Do	HCl (pH 2.4)	.002	43
Do	HCl, followed by water wash	.002	43
Do	CH_3COOH (pH 2.4)	.004	43
Do	CH_3COOH, followed by water wash	.005	41
Electrodialyzed	H_2SO_4 (pH 2.4)	.004	43

2. EFFECT OF PRETREATMENT WITH DIFFERENT CATIONIC SUBSTANCES

During processing, such as dyeing, silk is frequently treated with acidic solutions, which, as seen from the foregoing experiments, reduces the cation content and increases the susceptibility of the fiber to damage by light. The question whether stability of acid-treated silk can be increased by replacement of cations thus becomes of importance.

Preliminary experiments showed that basic inorganic substances, such as sodium hydroxide or sodium carbonate, are effective for increasing the stability of the silk, but they impart a harsh, undesirable feel to the cloth. It was observed, however, that basic organic compounds do not produce such undesirable effects, and accordingly, a few of these types of compounds were studied. The effect of pretreatment of samples of degummed and electrodialyzed or acid-treated silk with 0.01 *M* solutions of several cationic compounds is shown in table 2. The data show that the deleterious effect, on light exposure, produced by acidic treatments can be reversed by treatments of the silk with suitable reagents. It is seen that the stability of the silk to light increases with increase in pH of the solutions used for treatment of the silk. This is to be expected, since as shown by the titration curve of silk [6], the binding of cations also increases with increasing pH. In addition to pH, the chemical nature of the cationic substance may also influence the rate of the photochemical decomposition, but sufficient data are not available to enlarge upon this point.

In general, the inhibiting action of a given compound was independent of the history of a given sample; thus, degummed, acid-treated, or electrodialyzed silks all exhibited the same stability after treatment with a given reagent.

TABLE 2.—*Effect of different cationic substances on the deterioration of silk*

Sample	Treatment [a]	pH of solution	Loss in breaking strength on exposure to light
			%
Degummed			32
Acid-treated			41
Electrodialyzed			41
Degummed	Dodecyltrimethylammonium bromide (tech.)	6.0	32
Acid-treated	do	6.0	33
Degummed	do	9.8	24
Acid-treated	do	9.8	26
Degummed	Triethanolamine	10.4	26
Acid-treated	do	10.4	23
Electrodialyzed	do	10.4	26
Degummed	Trimethylbenzylammonium hydroxide	11.5	21
Acid-treated	do	11.5	19
Electrodialyzed	do	11.5	19
Degummed	Dimethyldibenzylammonium hydroxide	11.5	21
Acid-treated	do	11.5	21
Electrodialyzed	do	11.5	21

[a] The concentration of the solutions was 0.01 *M*. Treatment with more concentrated solutions gave no additional protection.

It was noted that the use of a cationic substance in the form of the salt, such as dodecyltremethylammonium bromide (tech.), had no effect on the degummed silk, although it did raise the stability of the acid-treated sample to that of the degummed sample. Addition of enough dilute sodium hydroxide to raise the pH of the salt solution to 9.8 produced a significant increase in the stability of both, although an equivalent amount of sodium hydroxide alone had no measurable effect on the silk. The reason for this is found in the titration studies of silk [6], in which it was shown that, at a given pH, silk combines with considerably more base in the presence of salts than in their absence.

When the samples which had been treated with the reagents listed in table 2 were washed thoroughly with distilled water, most of the inhibiting effects of the reagents were eliminated. The stabilities were lowered to about that of the original degummed silk, but they were still higher than those of the electrodialyzed or acid-treated samples. The pH of the water used for washing the samples was about 6.5, and accordingly, the silk would have still retained appreciable amounts of cations [6], which would account for these differences. It may be that Bonicatti's [4] failure to observe the stabilizing effect of cations resulted from his use of degummed silk which might have contained appreciable amounts of cation. This appears likely, since he found a marked decrease in the stability of his silk when he pretreated it with acid.

3. EFFECT OF METHYLATION

It is shown in the previous sections that the removal of cationic substances from silk by two independent methods results in practically identical decreases in stability to light, but that the stability can be restored by treatment with basic compounds. The question therefore arises whether the decreased stability obtained after electrodialysis or acid treatment is caused by the removal of cations from the carboxylic acid or the tyrosine hydroxyl groups, or from both.

It is not possible to ascertain by a simple experiment whether the cations are combined with carboxyl groups, tyrosine hydroxyl groups, or both. However, it is possible to inactivate known amounts of either type of group with diazomethane [7]. The results of such a procedure, applied to silk containing 0.002 milliequivalent, or less, of cation per gram, are shown in table 3. It was found that methylation of 5 percent of the hydroxyl groups produces a significant increase in the stability of the silk. Samples II, III, and IV were approximately equal with regard to the methylation of the hydroxyl groups and equal with regard to their stabilities to light, although they differed greatly with respect to the extent of methylation of their carboxyl groups. This shows that the methylation of the latter has no effect on the stability of the silk. However, a further increase in the methylation of the hydroxyl groups, from 17 to 87 percent of the total, produced still greater stability. The stabilities attained are comparable to the stabilities of those samples which were treated in alkaline solutions at pH 10.4 or above (table 2). Some additional evidence suggesting a confirmation of this explanation for an increase in the stability of silk on treatment in basic solutions was obtained in the following way. Portions of samples IV and V were treated with triethanolamine and trimethylbenzylammonium hydroxide, and the treated samples were then irradiated for 20 hours. As shown in table 4, treatment of sample V had little additional effect on stability. This was to be expected since most of the hydroxyl groups had already been methylated. On the other hand, the stability of IV after similar treatments was appreciably increased. In this sample, however, a large number of hydroxyl groups were still available for combination with the cations.

The increase in stability of silk, obtained by replacing the hydrogen of the hydroxyl group of tyrosine by methyl groups or by various cations, may result from a change in the absorption spectrum of the silk. The absorption spectra of some simple aromatic hydroxy-aldehydes and hydroxy-ketones are changed by similar treatments [11].

TABLE 3.—*Effect of selectively methylating carboxyl and tyrosine hydroxyl groups with diazomethane on the deterioration of silk*

Sample	Carboxyl	Tyrosine hydroxyl	Loss in breaking strength on exposure to light
	% of total	*% of total*	%
Electrodialyzed	0.0	0.0	41
I	0	5	35
II	15	16	31
III	31	15	31
IV	67	17	32
V	100	87	23

TABLE 4.—*Effect of cationic substances on the deterioration of samples of silk methylated to different extents*

Sample	Treatment	Loss in breaking strength on exposure to light
		%
IV	None	32
IV	Triethanolamine, pH 10.4	27
IV	Trimethylbenzylammonium hydroxide, pH 11.5	18
V	None	22
V	Triethanolamine, pH 10.4	25
V	Trimethylbenzylammonium hydroxide, pH 11.5	22

4. EFFECT OF THE IRRADIATION ON THE TYROSINE CONTENT OF SILK

The deterioration of silk fibers by photochemical reactions probably results from a photochemical fragmentation of the polypeptide chains of the protein [3]. Some of the tyrosine may be destroyed [12, 13] and peptide linkages adjacent to the tyrosine cleaved [14]. Destruction of some of the tyrosine was also noted in the present investigation, as shown by the data in table 5. It is of interest to note that the decrease in tyrosine content was accompanied by a corresponding decrease in tensile strength, and that treatments which tended to increase the stability of the silk decreased the amount of tyrosine destroyed.

TABLE 5.—*Photochemical decomposition of treated and untreated silk measured by change in tyrosine content and breaking strength*

Sample	Tyrosine	Loss in breaking strength on exposure to light
	%	%
Degummed (unexposed)	12.1	
Degummed+triethanolamine (pH 10.4)	12.0	33
Degummed+sodium hydroxide (pH 10.7)	11.5	53
Degummed	11.1	62
Acid-treated	10.9	77

5. PRACTICAL SIGNIFICANCE

The above observations show that the stability of silk to light can be considerably increased by treatment with suitable cationic reagents. Experiments with several organic bases show that the undesirable effects produced by inorganic bases can be avoided by the use of proper organic reagents. In addition, the latter type of compounds appears to be more efficient in regard to stabilizing the silk. It should be noted that, with the exception of the diazomethane treatment, none of the effects of the treatments used in this work was of a permanent nature, since the stabilizing effect was removed by washing the fabrics with water. It may be possible to develop colorless, basic compounds which might have sufficiently high affinities for the fiber so that they would resist removal by washing in neutral or alkaline solutions. Whether any of these treatments will affect

other properties of the fabric, such as light-fastness of the dye which may be used, can only be determined for the materials to which such reagents may be applied.

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ROLE OF CYSTINE IN THE STRUCTURE OF THE FIBROUS PROTEIN, WOOL

By Wilbur I. Patterson, Walton B. Geiger, Louis R. Mizell, and Milton Harris [1]

ABSTRACT

A number of the important physical and chemical properties of wool are closely associated with the presence of disulfide cross-linkages between the polypeptide chains of the protein. This conclusion results from a study of the behavior of wool before and after alteration of the mode of linkage of the sulfur by a series of highly specific reactions.

The cystine in wool can be readily reduced to cysteine with thioglycolic acid. Strongly alkaline solutions of the reagent have been shown by previous investigators to dissolve the protein and destroy its fibrous structure. It is now shown that wool can be reduced with thioglycolic acid over a wide range of pH, and that when the reduction is carried out in neutral or acid solution, the fibrous structure of the wool is not destroyed when the disulfide groups are reduced to sulfhydryl groups. The sulfhydryl groups of fibers reduced in this way react readily with alkyl halides to form thioether groups.

Thus reaction of reduced wool with alkyl monohalides, such as methyl iodide, results in permanent rupture of disulfide linkages, and the fibers are greatly increased in extensibility and decreased in strength. Alkylation with aliphatic dihalides, such as methylene iodide or trimethylene dibromide, introduces hydrocarbon chains between pairs of sulfur atoms of cystine molecules in the fibers. Such fibers are very similar to untreated fibers in physical properties.

Wool in which the disulfide linkages have been broken by reduction, or by reduction and alkylation with alkyl monohalides, possesses much higher alkali-solubility than untreated wool, while wool in which the disulfide cross-linkages have been replaced by new covalent cross-linkages through reduction followed by alkylation with dihalides possesses much lower alkali-solubility. Since the susceptibility of wool to degradation by alkalies is one of its greatest disadvantages, processes that would make it stable toward alkalies should also enhance its durability.

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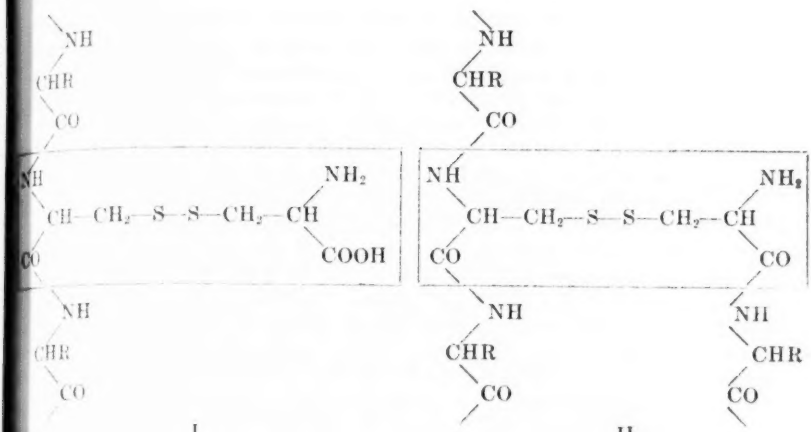
I. INTRODUCTION

Proteins, according to the hypothesis of Hofmeister [1][2] and Fischer [2], consist of amino acids joined through amide (peptide) linkages to form long chain structures called polypeptides. On the basis of this hypothesis, many of the similarities among proteins should be due to the large number of peptide linkages, while dissimilarities among them should be related to variations in the proportions and arrangement of the constituent amino acids, to differences in the lengths and arrangement of the polypeptide chains, and to the presence of linkages other than peptide. The present paper is concerned with the relationship of one such linkage, the disulfide linkage of the amino acid cystine, to the properties of the fibrous protein, wool.

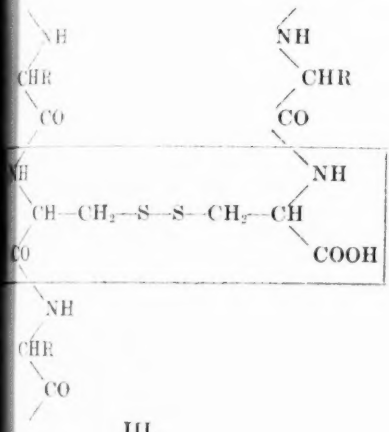
Although cystine contains two amino and two carboxyl groups, it appears that all of these groups are involved in peptide links in most, if not all, of the cystine residues. If the cystine of wool were combined in a peptide chain only through amino and carboxyl groups attached to the same carbon atom (formula I), processes that break disulfide bonds should result in the elimination from the wool of one-half of the sulfur. This possibility is not supported by experiment since reduced wool contains as much sulfur as the original material. Were either (or both) or the amino groups of cystine uncombined (formula II), treatment of wool with nitrous acid would destroy at least half of the cystine in the former case and all of it in the latter. The investigations of Hess and Sullivan [3], as well as unpublished work in this laboratory, show that only a small portion is destroyed by this reagent. Evidence derived from titrations [4, 5] indicates that no considerable number of carboxyl groups of cystine in wool can be free (formula III) since the number of free carboxyl groups is essentially accounted for by the content of glutamic and aspartic acids. While these data do not afford complete proof, they strongly indicate that all the functional groups of all the cystine are combined, and that only a small proportion, if any, can be free.

[1] Figures in brackets indicate the literature references at the end of this paper.



I.

II.



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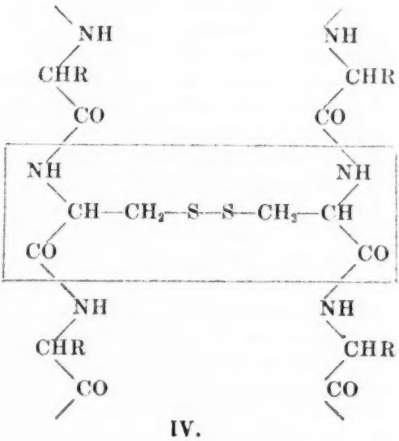

IV.

On the basis of these considerations, at least the major portion of the cystine must form parts of either two separate polypeptide chains (formula IV) or of a single folded polypeptide chain, and in either case its disulfide group would serve as a connecting or a cross-link. Astbury and Street [6] were the first to suggest that such disulfide cross-linkages were present in wool protein; since then Astbury, and Speakman, with their collaborators, have extended this hypothesis.

This view suggests that modification of the mode of linkage of the sulfur in wool should lead to profound alterations in the properties of the fiber. Unfortunately, since many of the processes known to affect disulfide linkages, such as the action of light, of alkalies, and of oxidizing and of some reducing agents, involve side reactions with other groups in the protein, conclusions based on their use may be open to criticism. The action of a few reducing agents, however, appears to be solely with the disulfide groups and therefore it appeared possible to use these reagents in evaluating the relation of the disulfide groups to the structure and properties of the wool fiber. Of these reagents, only thioglycolic acid and certain other mercaptans can reduce each disulfide group to two sulfhydryl groups. Other reagents, such as potassium cyanide, sodium sulfide, and sodium bisulfite, react with disulfide groups to form not only sulfhydryl groups but others as well [7].

Goddard and Michaelis [7, 8] found that strongly alkaline solutions of thioglycolic acid rapidly converted the disulfide groups of wool to sulfhydryl groups, and that under these conditions, rapid dissolution of the wool occurred. These authors used alkaline solutions since they observed that wool was not dissolved by neutral or acid solutions of the reagent, an observation that led them to conclude that wool could be reduced only at a pH of 10 or higher. Such a conclusion is not compatible with the behavior of disulfides in general, which are known to be readily reduced over a wide range of pH values. In the present investigation, it is shown that the disulfide groups of wool can also be reduced by thiogycolic acid over a wide range of pH, and that if the reduction is carried out in neutral or acid solutions, the fibrous structure of the wool is not destroyed. The use of neutral or faintly acidic solutions of the reducing agent also has the advantage of eliminating such secondary reactions as degradation of cystine, cysteine, peptide, or other linkages.

Wool that has been reduced in neutral or acid solution can be further modified without loss of fibrous structure by treatment with alkyl halides, which convert the sulfhydryl groups to thioether groups.[2] The reactions may be presented as follows:

$$W-S-S-W+2HS-CH_2-COOH \longrightarrow 2W-SH+(S-CH_2-COOH)_2 \quad (1)$$

$$W-SH+RX \longrightarrow W-SR+HX, \quad (2)$$

where W represents the portions of the wool connected by the disulfide groups, R represents an alkyl group, and X, a halogen atom. Reactions of this type result in the permanent rupture of covalent cross-linkages. Wool fibers have been prepared in which the mode of

[2] Goddard and Michaelis [7, 8,] and Pillemer, Ecker and Wells [9, 10], also found that their amorphous wool protein derivative could be alkylated by treatment with alkyl monohalides to form other amorphous protein derivatives.

linkage of at least 95 percent of the cystine has been modified through these reactions.

Dihalides are also capable of reacting with the reduced wool, the reaction being represented by the equation

$$2W-SH+(CH_2)_nX_2 \longrightarrow W-S-(CH_2)_n-S-W+2HX. \quad (3)$$

The reaction with the dihalides results in the formation of new cross-links [4] in which the sulfur atoms of the cystine are connected by short hydrocarbon chains.

The reactions in eq 2 and 3 offered a means of preparing a large number of wool derivatives. Thus it was possible not only to investigate the nature of the sulfur linkages and their relation to the structure of the fiber but also to study the effects of introducing into the fiber new groups having widely different properties.

II. EXPERIMENTAL PROCEDURE

1. MATERIALS

The wool fibers used in the present study were part of the lot used in earlier investigations reported from this laboratory [12]. This material had been subjected to no mechanical or chemical treatment other than successive extractions with alcohol and with ether at room temperature for 5 hr each, followed by washing with water at 40° C.

Worsted yarn was also used for some experiments. It was prepared from raw wool that had been previously extracted with Stoddard solvent and washed with water. The yarn was further purified by extraction in a Soxhlet apparatus with alcohol and with ether for 6 hr each, and finally washed with distilled water.

2. METHODS

(a) REDUCTION OF WOOL WITH THIOGLYCOLIC ACID

Aqueous solutions of thioglycolic acid were used, and were adjusted to the desired pH by adding potassium hydroxide solution. The final concentration of thioglycolic acid was determined by titration with standard iodine solution [13]. It was found advisable to cool the thioglycolic acid solution in an ice bath during addition of the potassium hydroxide, since at higher temperatures the solutions often became colored. Although aqueous solutions of thioglycolic acid were used in all experiments reported in this paper, solutions of the reagent in organic solvents were also found effective.

The reduction was carried out by immersing a known weight of wool in a solution of thioglycolic acid at the required temperature, pH, and concentration. Wetting the wool was facilitated by evacuating the vessel with a filter pump. After the reduction had been allowed to proceed for the desired length of time, the wool was removed and washed several times with distilled water to remove excess thioglycolic acid and its oxidation products.

[4] Speakman [11] has suggested that divalent metal ions, such as that of barium, can form cross-linkages between sulfur atoms. Such linkages would be ionic and unstable, as compared with completely homopolar linkages formed according to eq 1 and 3.

(b) REOXIDATION OF SULFHYDRYL GROUPS OF REDUCED WOOL

Before the reduced wool was reoxidized, it was first washed thoroughly with 95-percent ethyl alcohol. Omission of this step led to products that contained amounts of sulfur in excess of that in the untreated wool, presumably by the formation of a mixed disulfide, one-half derived from cysteine, the other half from thioglycolic acid. The wool was then rinsed with distilled water, placed in 0.1 *M* potassium phosphate buffer solution at pH 7.0, and a stream of oxygen bubbled through the solution until the wool no longer gave a positive nitroprusside reaction [14]. Finally, the wool was again washed with distilled water, and dried in a stream of air.

(c) ALKYLATION OF SULFHYDRYL GROUPS OF REDUCED WOOL

Reduced wool that was to be alkylated was transferred immediately after washing to a 1.0 *M* phosphate buffer solution at pH 8.0, in which had been dissolved or suspended the desired alkylating agent. The rate of alkylation was increased by agitation of the mixture, particularly when the alkylating agent was not completely soluble. Ordinarily, 0.002 mole of alkylating agent and 100 ml of buffer solution were used for each gram of wool. The reaction was allowed to proceed until a negative nitroprusside test for sulfhydryl groups in the fiber was obtained.

The reactions take place not only in aqueous buffer solutions but also in organic solvents in the presence of soluble or insoluble substances capable of removing the acid formed in the reaction. For example, solutions of reagents in 50-percent aqueous methanol, 0.05 *M* with respect to NH_4OH and 0.1 *M* with respect to NH_4Cl, or in benzene in the presence of sodium bicarbonate, were found to be satisfactory.

(d) ANALYTICAL METHODS

The extent to which the sulfhydryl groups of the reduced wool had been alkylated was determined by analyzing the wool for cystine by the Sullivan [15] procedure, as previously used in this laboratory [16]. Neither thioglycolic acid nor thioethers derived from cysteine affect the color reaction of the cystine under these conditions. The adequacy of this method of determining the amount of reaction was checked in certain cases by alkylating the reduced wool with *p*-chlorobenzyl chloride, a reagent that introduces an atom of halogen for each sulfhydryl group with which it reacts. For example, one product that contained 2.2 percent of chlorine (0.0C063 equivalent per gram), also was found to contain 4.7 percent of cystine. The original wool contained 12.4 percent of cystine, so allowing for the increase in weight due to introduction of *p*-chlorobenzyl groups during the reaction, the product contained a calculated total of 11.4 percent of reacted plus unreacted cystine. By subtracting the 4.7 percent of unreacted cystine from this total, it is seen that 6.7 percent, or 0.00056 equivalent, of cystine in each gram of wool had reacted. The amount of halogen introduced therefore corresponds, within analytical error, with the decrease in cystine content of the wool.

The amount of halogen was determined by the Pregl bead-tube method [17]. Sulfur determinations were made by the oxygen-bomb method [18].

The hygroscopicity [5] of the wool was determined by keeping air-dried samples in a room at 21° C and 65-percent relative humidity for 24 hr, and determining the loss in weight on drying in a vacuum oven at 105° C for 2 hr. The values are calculated as percentages of the dry weights.

Alkali-solubility was measured by the method of Harris and Smith [19]. Samples were treated with 0.1 *N* sodium hydroxide solution for 1 hr at 65° C, and the percentage loss in weight was determined.

(e) DETERMINATION OF PHYSICAL PROPERTIES OF THE WOOL

Two tests were utilized in determining the changes produced in the physical properties of the fiber during the various treatments. The first involved the measurement of the breaking strength of treated yarn by the single-strand method on a pendulum-type machine. The values reported are the averages of 10 or more determinations.

In the second test, the stress-strain characteristics of individual fibers were determined by the method described by Sookne and Harris [12]. This method, which is a modification of procedures used by other investigators.[6] is much more sensitive to small changes and has greater accuracy than methods involving a determination of the breaking strengths. It is based upon the observation of Speakman [5] that when a wool fiber is immersed in water and stretched under load until it has been elongated 30 percent, it will return practically to its original length when the load is removed. If the fiber is allowed to relax in the unstretched state in water for about 24 hr., and then again stretched exactly as before, it is found that the same amount of energy (within about 1 percent) is required to stretch the fiber the second time. If the fiber after the first stretching is subjected to chemical treatments which break chemical bonds, less energy will be required to stretch it the second time. The ratio of the energy required to stretch the fiber the second time to that required the first time is called the *30-percent index.* It follows that if the 30-percent index is less than 1, the fiber has been weakened by the treatment; if it is greater than 1, strengthened. The accuracy of this method depends on the fact that comparisons are always made between successive measurements on the *same* fiber. Since wool fibers are extremely irregular and vary considerably in shape and cross section, comparisons between *different* fibers would give rise to large errors, which could be only partially eliminated by the application of statistical methods to measurements on large numbers of fibers. With the present technique, as few as three fibers were found sufficient to provide adequate checks.

III. RESULTS AND DISCUSSION

1. COURSE OF THE REDUCTION REACTION

The reaction of wool with thioglycolic acid under a variety of conditions was investigated and is described in the following sections.

[5] The term "hygroscopicity" is used here in an arbitrary way. Actually, the amount of moisture absorbed by wool will vary both with temperature and relative humidity.

[6] For literature citations, see [12].

(a) EFFECT OF pH

Preliminary work showed that solutions of thioglycolic acid to which no alkali had been added reacted with wool, and resulted in the production of sulfhydryl groups, which gave a positive nitroprusside reaction, and were capable of reacting with alkyl halides. These reactions proceeded without destruction of the fiber, whereas strongly alkaline solutions (pH 11.0 or above) of the reagent reacted with the wool, not only to reduce it, but also to dissolve it [7, 8]. The effect of various pH values was studied in greater detail by reducing several samples of wool with a 0.2 *M* solution of thioglycolic acid at 35° C for 20 hr., then alkylating with *p*-chlorobenzyl chloride, and finally determining the amount of unchanged cystine. The results are shown in figure 1. It will be noted that the amount of reduction was practically constant over the pH range of 2 to 6, somewhat greater at pH 8, and very markedly greater at pH 10. The decrease in cystine in the alkaline region is due not to destruction of cystine by alkali but to reduction, since wool that has been reduced for short periods (2 hr) under these conditions can be reoxidized to products containing nearly the original amounts of cystine and sulfur. Longer exposure (20 hr) of wool to thioglycolic acid at pH 10 resulted in material that contains an abnormally low amount of cystine after reoxidation, but because a considerable portion of the wool was dissolved under such conditions, the results were probably caused by extraction of cysteine-rich material. The increased reduction in alkaline solutions may be caused by ionization of sulfhydryl groups. This could change the mechanism of action of the reducing agent, and also increase swelling and facilitate penetration of the reagent.

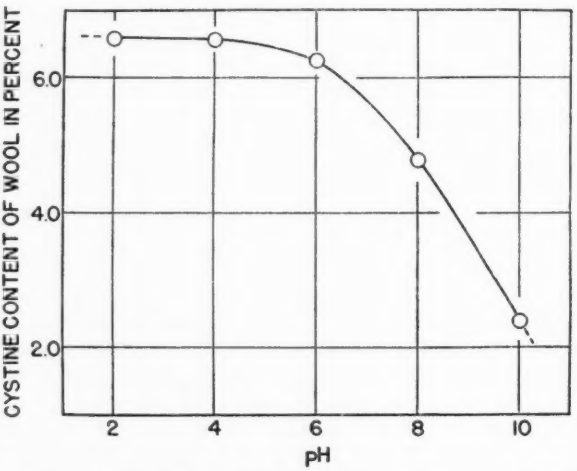

FIGURE 1.—*Cystine content of wool after reduction at various* pH *values for 20 hours at 35° C with 0.20 M thioglycolate solutions, and alkylation with p-chlorobenzyl chloride.*

Wool reduced in acid solution maintained its original appearance, but wool reduced at pH values of 9.0 or above became gelatinous when wet, and brittle and resinous when dry.

(b) EFFECT OF THE DURATION OF THE TREATMENT

The amount of cystine reduced during treatment of wool for various lengths of time with 0.2 *M* thioglycolic acid at pH 4.5 and 35° C is shown in figure 2. Under these conditions the reaction proceeded to

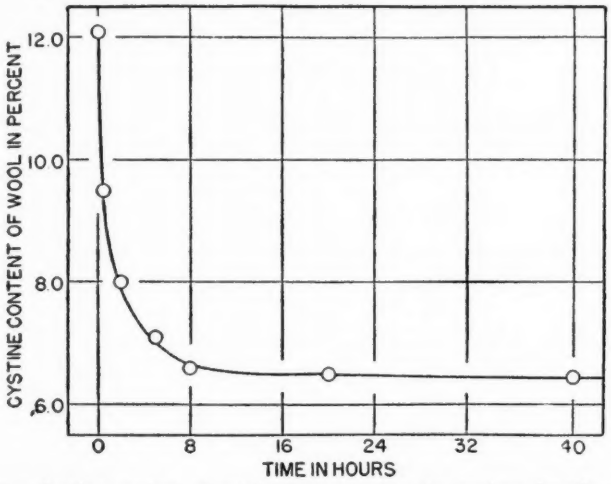

FIGURE 2.—*Cystine content of wool after reduction for various lengths of time at* pH *4.5 and 35° C with 0.2 M thioglycolate, and alkylation with p-chlorobenzyl chloride.*

about one-half of its final extent in the first 2 hr, and to about 95 percent of its final extent in 8 hr.

(c) EFFECT OF THE CONCENTRATION OF THIOGLYCOLIC ACID

The amount of cystine reduced in 20 hr at 35° C and at pH 4.5 by various concentrations of thioglycolic acid is indicated in figure 3.

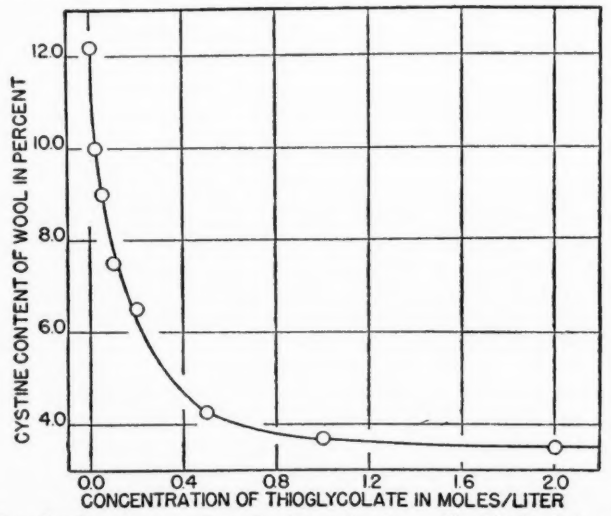

FIGURE 3.—*Cystine content of wool after reduction with thioglycolate solutions of various concentrations for 20 hours at* pH *4.5 and 35° C, and alkylation with p-chlorobenzyl chloride.*

Sufficient reducing reagent to insure the presence of at least 20 times the amount of thioglycolic acid theoretically necessary to reduce all the disulfide groups of the wool was used in each experiment. The amount of reduction increased rapidly in the range 0.01 to 0.2 *M*. The effect was less marked between 0.2 and 0.5 *M*. Increasing the concentration of thioglycolic acid beyond 0.5 *M* had little further effect.

(d) EFFECT OF TEMPERATURE

The amounts of cystine reduced during treatment of wool for various lengths of time with 1.0 *M* thioglycolic acid at pH 4.5 and at 10° and 50° C are shown in figure 4. At 50° the reaction was essentially

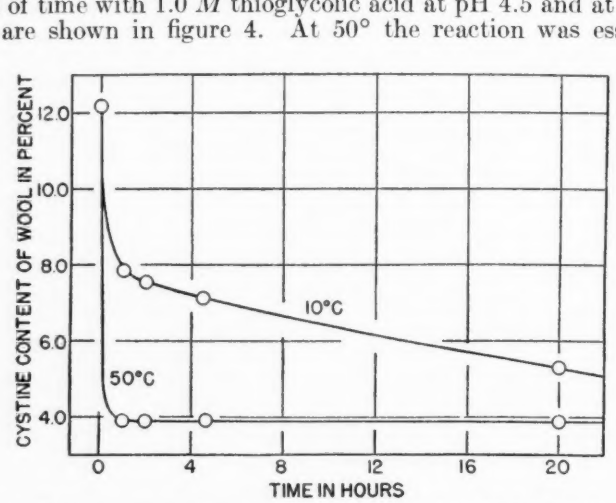

FIGURE 4.—*Cystine content of wool after reduction with 1.0 M thioglycolate at 10° C and at 50° C at* pH *4.5 for various lengths of time, and alkylation with p-chlorobenzyl chloride.*

complete after 1 hr, whereas at 10° the reaction proceeded more slowly. Increasing the temperature did not increase markedly the amount of cystine finally reduced.

2. ALKYLATION

Most of the alkyl halides that were investigated were found to react more or less readily with reduced wool. In general, the reaction took from 6 to 24 hr at 35° C to reach completion, depending on the amount of reduction of the wool and on the reagent used. It was found that iodides reacted faster than bromides, and bromides faster than chlorides. Tertiary alkyl halides, such as tertiary butyl chloride and triphenylmethyl chloride, were found to be almost completely unreactive.

Besides the reagents listed in table 1, the following were also found to be reactive: allyl chloride, 2-methylallyl chloride, chloroacetone, methyl chloromethyl ether, ethyl chloride, ethylene dichloride, ethylene oxide, epichlorohydrin, 1,3-dichloroisopropanol, and 1,3-dibromoisopropanol.

3. PROPERTIES OF REDUCED AND ALKYLATED WOOLS

The properties of wool fibers and yarn that had been modified by reduction and alkylation are summarized in the following sections. In each instance, the wool was reduced for 20 hr at 35° C and pH 4.5 with a large excess of 0.2 *M* thioglycolate. Duplicate experiments were found to lead to variations amounting to less than 0.02 in the 30-percent indices, 0.3 percent in hydrations, and 0.5 percent in alkali-solubilities. Analyses showed that the materials contained 6.5±0.5 percent of cystine in every case; that is, the mode of linkage of approximately half of the sulfur atoms had been altered, since the untreated wool contained 12.4 percent of cystine.

TABLE 1.—*30-percent indices and breaking strengths of wools reduced and alkylated with alkyl monohalides*

Treatment	30-percent index	Breaking strength
		Grams
Untreated	0.99	1,310
Reduction	.65	1,170
Reduction and reoxidation	.97	1,310
Reduction and CH_3I	.67	1,190
Reduction and C_2H_5Br	.70	1,250
Reduction and $C_6H_5CH_2Cl$	.92	1,310
Reduction and p-$ClC_6H_4CH_2Cl$	.95	1,450
Reduction and $C_7H_{15}Br$	.95	1,380
Reduction and $C_{12}H_{25}Br$	.96	1,340
Reduction and $ClCH_2COOH$	.67	1,240

(a) PHYSICAL PROPERTIES

As indicated by table 1, the breaking of cross-linkages during reduction greatly decreased the 30-percent index of the fibers. Reduction followed by reoxidation resulted in the restoration of nearly the original 30-percent index, indicating practically complete reaction of the sulfhydryl groups to rebuild disulfide cross-linkages. Fibers modified by reduction followed by alkylation with an alkyl monohalide of low molecular weight, such as methyl iodide or ethyl bromide, were only slightly less easily elongated than those reduced alone. Alkylation with chloroacetic acid introduced ionizable carboxyl groups (combined as $-S-CH_2COOH$) into the fiber. The data show that wool fibers treated in this manner have 30-percent indices of the same order as those of wool fibers reduced alone.

Reduction of wool followed by alkylation with monohalides of higher molecular weight, such as benzyl chloride, *p*-chlorobenzyl chloride, heptyl bromide, or dodecyl bromide gave products higher in 30-percent index than those obtained with methyl iodide or ethyl bromide. Such behavior appears to be due either to the action of intermolecular forces of the van der Waals type between alkyl groups on neighboring sulfur atoms, or between these groups and other parts of the polypeptide chains of the protein. Such secondary linkages would be expected to be weaker than the original covalent type. The data in table 1 offer support for such a hypothesis.

When aliphatic dihalides are used to alkylate reduced wool, groupings that may be represented as $W-S-(CH_2)_n-S-W$ appear to replace the disulfide bridges of the original wool, as eq 3 indicates. Analogous reactions of dihalides with simple mercaptans are known [20], and evidence that the reaction proceeds in this way is found both

in the properties of the products, and in the fact that they are practically free of halogen. The halogen contents of several samples of wool prepared by reduction and alkylation with trimethylene dibromide are shown in table 2. Included in the table are halogen contents calculated on the basis of the assumption that only one of the bromine atoms of the dihalide had reacted. It is readily seen that only a very small fraction of the dihalide molecules had reacted in this way. Extensibility of the wool prepared in this way would be expected to be slightly increased, presumably since the cross-linkages between polypeptide chains would be lengthened, and it should increase further as the number of methylene groups separating the two sulfur atoms is increased. The data obtained with fibers reduced and treated with methylene iodide, ethylene dibromide, trimethylene dibromide, and tetramethylene dibromide show such a trend (table 3).

TABLE 2.—*Bromine content of wool reduced and alkylated with trimethylene dibromide*

Calculated results are based on the assumption that only one bromine atom of each dihalide molecule has reacted.

Cystine reacted	Bromine (calculated)	Bromine (found)
Percent	*Percent*	*Percent*
2.1	1.6	0.1
6.4	5.0	.1
8.4	6.5	.0
10.2	7.9	.2

TABLE 3.—*30-percent indices and breaking strengths of wools reduced and alkylated with alkyl dihalides*

Treatment	30-percent index	Breaking strength
		Grams
Untreated	0.99	1,370
Reduction and CH_2I_2	.94	1,380
Reduction and $(CH_2)_2Br_2$	.92	1,420
Reduction and $(CH_2)_3Br_2$	.90	1,460
Reduction and $(CH_2)_4Br_2$	.88	1,380

The breaking strengths of yarn treated like the fibers show similar trends (tables 1 and 3). Reduced wool, reduced and methylated wool, and wool reduced and treated with chloroacetic acid were markedly lower in breaking strength than the untreated wool, while wool that had been reduced and alkylated with monohalides of high molecular weight, or with a dihalide, was not far different from untreated wool in this respect.

(b) HYGROSCOPICITY

The hygroscopicity of the modified wools varied depending on the chemical nature of the groups introduced into the wool, as shown in tables 4 and 5. Wools into which large groups have been introduced by alkylation with benzyl chloride, *p*-chlorobenzyl chloride, heptyl bromide, or dodecyl bromide were found to be less hygroscopic than untreated wool. By introducing hydrophobic groups of high molecular weight into the wool the percentage of hydrophilic structures, such as peptide bonds, is decreased, and as a result the products are less highly hydrated.

TABLE 4.—*Alkali-solubility and hygroscopicity of wools reduced and alkylated with monohalides*

Treatment	Alkali-solubility	Moisture content
	Percent	*Percent*
Untreated	10.5	16.0
Reduction	cir. 50.0	
Reduction and reoxidation	12.5	16.3
Reduction and CH_3I	33.0	16.3
Reduction and C_2H_5Br	18.3	16.2
Reduction and $C_6H_5CH_2Cl$	15.4	14.8
Reduction and p-$ClC_6H_4CH_2Cl$	12.8	14.0
Reduction and $C_7H_{15}Br$	18.0	15.6
Reduction and $C_{12}H_{25}Br$	18.2	15.6
Reduction and $ClCH_2COOH$	cir. 50.0	16.9

TABLE 5.—*Alkali-solubility and hygroscopicity of wools reduced and alkylated with dihalides*

Treatment	Alkali-solubility	Moisture content
	Percent	*Percent*
Untreated	10.5	16.0
Reduction and CH_2I_2	5.2	16.4
Reduction and $(CH_2)_2Br_2$	6.6	16.2
Reduction and $(CH_2)_3Br_2$	5.3	16.0
Reduction and $(CH_2)_4Br_2$	7.0	16.3

The introduction of small groups by alkylation with methyl iodide, ethyl bromide, or an alkyl dihalide of low molecular weight should lead to introduction of only a small percentage of hydrophobic groups. As would be expected, these products were found to be very little different from untreated wool in hygroscopicity. The introduction of a polar group, as by reduction and alkylation with chloroacetic acid, yielded products that were somewhat more hygroscopic than untreated wool.

(c) ALKALI-SOLUBILITY

Reduction of wool, followed by alkylation with an alkyl monohalide may be expected to render the wool more readily soluble in alkali, since this process breaks disulfide bonds which form links between polypeptide chains, and therefore decreases the average molecular weight of the wool proteins. The high alkali-solubilities of wool that has been reduced and treated with alkyl monohalides illustrate such a behavior. The extremely high alkali-solubility of wool alkylated with methyl iodide may be due to the formation of sulfonium groupings according to the equation

$$WSCH_3 + CH_3I \longrightarrow W-S(CH_3)_2^+ + I^-.$$

Methyl iodide is known to be exceptionally reactive in this respect [21]. Sulfonium compounds are highly ionized and would be expected to increase the alkali-solubility.

Reduction breaks disulfide cross-linkages, a process which lowers the molecular weight and at the same time introduces ionizable sulfhydryl groups into the wool. Each of these changes should tend to increase the solubility of the wool in alkali, and observations confirmed this expectation. In view of these results, it is apparent why strongly alkaline solutions of thioglycolic acid, as observed by Goddard and Michaelis [7, 8], are capable of dissolving wool. Reduction followed

by alkylation with chloroacetic acid should also yield products that possess high alkali-solubility, since carboxyl groups also are ionized in alkaline solution. Such wool was found to be very readily dissolved by alkali.

Reduction of wool, followed by treatment with polymethylene dihalides such as methylene iodide, ethylene dibromide, trimethylene dibromide or tetramethylene dibromide, is presumed to maintain the original molecular weight of the protein, and also replaces disulfide cross-linkages, which are unstable toward alkali [22], with stable *bis*-thioether [7] cross-linkages as 3 indicates. Such a process was found greatly to increase the resistance of wool to the action of alkali. The alkali-solubilities (table 5) of these samples were much lower than those of untreated wool, or of any of the wools reduced and then alkylated with alkyl monohalides.

(d) EFFECT OF VARYING THE EXTENT OF REDUCTION AND ALKYLATION

The results described in the preceding sections were obtained from wool that had been reduced to nearly the same cystine content and then alkylated. The relation between the extent of reduction and alkylation, as judged from the decrease in content of unchanged cystine and the properties of the products, has also been studied, using two of the alkylating agents, methyl iodide and trimethylene dibromide. The experimental results are summarized in table 6.

It is apparent that in both cases the 30-percent index decreased as the extent of reduction and alkylation increased. Fibers that had been reduced and methylated, however, were decidedly more changed in this respect than those which had been reduced and treated with trimethylene dibromide. The breaking strengths of yarn show the same trend as the 30-percent indices of fibers treated in the same way, but are a much less sensitive means of detecting the changes that have taken place.

TABLE 6.—*Properties of wool reduced to indicated cystine content and alkylated with indicated reagents*

Cystine	30-percent index	Breaking strength	Alkali-solubility
(A) Untreated			
Percent 12.2	0.99	*Grams* 1,310	*Percent* 10.5
(B) Alkylated with $(CH_2)_3Br_2$			
10.3	0.99	1,338	5.8
6.0	.86	1,318	3.7
4.0	.72	1,272	2.6
2.2	.68	1,012	2.5
(C) Alkylated with CH_3I			
10.3	0.73	1,287	13.2
6.0	.48	1,250	31.5
4.0	.27	1,240	43.5
2.2	.18	989	83.0

[7] This terminology is in accord with the suggestions of Patterson and Curran [23].

Of particular interest is the fact that, while the alkali-solubility of the reduced and methylated wool increased as its content of unchanged cystine decreased, that of the wool reduced and treated with trimethylene dibromide decreased. This contrasting behavior furnishes further evidence that reduction followed by methylation breaks disulfide cross-linkages and decreases the average molecular weight of the wool protein, while reduction followed by treatment with trimethylene dibromide maintains the molecular weight by rebuilding cross-linkages of a type that are extremely stable to alkali.

Susceptibility to degradation by alkali, which has been one of the most important practical disadvantages of wool, can now be overcome to a large extent through conversion of relatively unstable disulfide cross-linkages to extremely stable thioether cross-linkages. The stability of wools of this sort, under a variety of conditions of practical interest, is now being investigated in this laboratory.

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